Universidade de Aveiro Departamento de Biologia
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Universidade do Porto Faculdade de Ciências

Isabel Maria Alves Natividade Campos

Contaminantes nas cinzas e solos após incêndios florestais e os seus efeitos a jusante

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências do Mar e Ambiente, realizada sob a orientação científica do Doutor Jan Jacob Keizer, Equiparado a Investigador Auxiliar do CESAM e Departamento de Ambiente e Ordenamento da Universidade de Aveiro. A coorientação foi realizada pelo Doutor Nelson José Cabaços Abrantes, Investigador Auxiliar do CESAM e Departamento de Ambiente e Ordenamento da Universidade de Aveiro, e pela Doutora Patrícia Alexandra Oliveira Pereira Kowalski, investigadora em Pós-Doutoramento do CESAM e Departamento de Biologia da Universidade de Aveiro.

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“A natureza só utiliza os fios mais longos para tecer os seus padrões, pelo que cada pequena parte do seu tecido revela a organização de toda a tapeçaria.”
Richard Feynman (1918-1968).

“All substances are poisonous, there are none which is not a poison; the right dose is what differentiates a poison from a remedy.”
Paracelsus (1493-1541).

o júri

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palavras-chave

Fogos florestais, Metais, Hidrocarbonetos aromáticos policíclicos (HAPs), Plantações florestais, Solos, Cinzas, Escorrências, Efeitos ecotoxicológicos, Espécies aquáticas.

resumo

Os incêndios florestais tornaram-se, nas últimas décadas, uma grande preocupação social e ambiental nas regiões de clima tipo-Mediterrâneo em todo o mundo, incluindo Portugal, devido ao aumento da sua frequência, severidade e extensão de áreas ardidas. Estas preocupações são ainda mais agravadas pelos cenários de alterações climáticas que preveem um aumento das condições propícias à ocorrência de incêndios.

Enquanto que os efeitos dos incêndios na vegetação e processos hidrológicos do solo estão bem documentados, o seu impacto na produção e mobilização temporal de contaminantes em cinzas e solos queimados e dos seus efeitos nos sistemas aquáticos a jusante, tem sido pouco estudado. Em particular, a libertação pós-incêndio de metais e hidrocarbonetos aromáticos policíclicos (HAPs) para o meio ambiente, a qual assume particular preocupação devido à sua toxicidade, persistência e tendência para a bioacumulação, com impactos potencialmente nocivos no ambiente assim como na saúde humana, permanecem uma lacuna importante da investigação.

Neste contexto, este trabalho pretende ser um contributo para compreender e avaliar o papel dos incêndios florestais na mobilização temporal de metais e HAPs, assim como dos seus efeitos a jusante. Deste modo, foi adotada uma abordagem integrativa para atingir estes objectivos. Primeiramente, quantificaram-se os níveis de vários metais (V, Mn, Co, Ni, Cu, Cd, Hg e Pb) e de quinze HAPs prioritários, em cinzas e solos não queimados e queimados recolhidos em plantações florestais no Centro-Norte de Portugal. Estas cinzas e solos foram recolhidos imediatamente após o incêndio, assim como quatro (após o primeiro evento de chuva pós-incêndio), oito e quinze meses após o incêndio. Foi também avaliado em que medida o tipo de floresta (plantações de eucalipto versus pinho) refletia os teores de contaminantes nas amostras analisadas. Por fim, foi realizada uma avaliação ecotoxicológica para investigar os efeitos tóxicos das escorrências superficiais recolhidas imediatamente e um ano após o incêndio. Esta avaliação foi efectuada com quatro espécies standard, representando diferentes grupos funcionais e níveis tróficos.

De um modo geral, os resultados deste estudo indicaram um aumento consistente dos níveis de metais e HAPs em solos queimados de eucalipto em comparação com os solos de eucalipto não queimados. Estes incrementos apontaram para uma incorporação destes elementos nos solos queimados durante ou após o incêndio. Contrariamente, os resultados mostraram que 30 % do Hg retido nos solos de eucalipto foi libertado pelo incêndio, correspondendo a uma perda de 1.0 - 1.1 g Hg ha^{-1}. Além disso, as cinzas apresentaram-se sempre enriquecidas em metais e HAPs em relação aos solos subjacentes, sugerindo que as concentrações de metais e HAPs na camada superficial do solo foram influenciadas pela deposição das cinzas resultantes do incêndio florestal. Adicionalmente, as cinzas e os solos queimados caracterizaram-se por elevados níveis de HAPs com três e quatro

anéis, no entanto, o perfil de composição diferiu entre os solos queimados e não queimados. A distribuição dos HAPs pelo número de anéis e as razões de diagnóstico apontaram para que o incremento de HAPs nos solos queimados tenham sido maioritariamente originados pela queima de biomassa. As cinzas e os solos das encostas de eucalipto apresentaram níveis mais elevados de Hg relativamente às áreas de pinho, enquanto que o oposto se verificou para os teores de Co, Ni e HAPs. O tempo decorrido após o incêndio influenciou o comportamento dos metais e HAPs totais de três modos distintos: diminuição abrupta nas concentrações de Mn, Cd e HAPs após os primeiros eventos de chuva pós-incêndio (quatro meses após o incêndio); aumento dos níveis de V, Ni e Co durante os primeiros oito meses após o incêndio; e os níveis de Cu, Hg e Pb mantiveram-se praticamente inalterados durante todo o período de estudo. Além sido, as cinzas apresentaram picos de concentração em metais e HAPs imediatamente após o incêndio, registando-se uma diminuição brusca quatro meses depois. Esta diminuição poderá estar relacionada com a precipitação intensa ocorrida e a geração de escorrência superficial que terá promovido a lavagem e a lixiviação destes elementos das cinzas e dos solos.

As escorrências superficiais apresentaram valores de HAPs significativamente mais elevados (quatro vezes) após o primeiro evento de chuva pós-incêndio do que um ano mais tarde. Do mesmo modo, verificaram-se diferenças na composição em termos de anéis e em termos de HAPs individuais. Ambas as amostras de escorrências superficiais induziram uma redução no crescimento dos dois produtores primários - *Pseudokirchneriella subcapitata* e *Lemna minor* – e inibiram a luminescência da bactéria *Vibrio fischeri*. Contrariamente, o invertebrado - *Daphnia magna*, não foi significativamente afetado nos respetivos ensaios. Surpreendentemente, a amostra de escorrência superficial recolhida um ano após o incêndio revelou ser a mais tóxica para as espécies estudadas.

No geral, o presente trabalho contribui com uma nova perspetiva sobre o papel dos incêndios e as chuvas subsequentes na mobilização de metais e HAPs de solos e cinzas de áreas florestais ardidas, os quais constituem uma importante fonte difusa de poluição. Adicionalmente, este trabalho também enfatiza os riscos apresentados por estes contaminantes para os sistemas aquáticos a jusante, com consequências deletérias em ambas as suas condições química e biológica.

Tendo isto em consideração, este estudo poderá ser um ponto de partida para a conceção e implementação de futuros programas de monitorização de áreas ardidas como parte dos planos de gestão pós-incêndio.

keywords

Forest fires, Metals, Polycyclic aromatic hydrocarbons (PAHs), Forest plantations, Soils, Ashes, Runoff, Ecotoxicological effects, Aquatic species.

abstract

The increased frequency, severity and extent of wildfires over the past decades have become a major societal and environmental concern in Mediterranean-type climate regions across the world, including Portugal. These concerns are further aggravated by the likely future climate conditions, increasingly propitious to wildfire ignition and spreading.

Whilst the impacts of wildfires on vegetation and soil hydrological processes have received considerable research attention, little is known about contaminants in ashes and burnt soils, their production and mobilization with time-since-fire and their off-site effects on aquatic systems. In particular, the fire-induced release of major and trace elements (metals) and polycyclic aromatic hydrocarbons (PAHs) into the environment, which assumes a singular concern due do their toxicity, persistence and tendency to bio-accumulate, with potentially harmful impacts on the environment as well as human health, has been neglected.

Hence, the present work aims to be a contribution to understand and evaluate the role of wildfire and time-since-fire in the mobilisation of metals and PAHs and their off-site effects. As a way to fulfil these proposes an integrative approach was adopted. First, the levels of metals (V, Mn, Co, Ni, Cu, Cd, Hg and Pb) and the fifteen priority PAHs were surveyed in ashes and burnt and unburnt soils collected immediately after a wildfire in forest plantations in north-central Portugal, as well as four (after the first post-fire rainfall events), eight and fifteen months later. It was also investigated in what extent the forest type (eucalypt versus pine plantations) constrain the levels of the contaminants. Then, to assess the toxic effects of the surface runoff collected immediately after fire and one year later, an ecotoxicological assessment was performed using four standard species representing different functional groups and trophic levels.

In general, results of this study showed a consistent increase in the levels of metals and PAHs in burnt eucalypt soils compared to unburnt eucalypt soils. The inputs of these contaminants point to an incorporation of these elements in burnt soils during or after the fire. However, results showed that 30 % of the Hg retained in the eucalypt soils were released by the wildfire, corresponding to a loss of 1.0-1.1 g Hg ha^{-1}. Moreover, ashes were consistently enriched in metals and in PAHs relatively to the underlying topsoil, suggesting that concentrations of metals and PAHs in topsoil were influenced by the deposition of ash resulting from the wildfire. Additionally, ashes and burnt soils were characterized by higher levels of PAHs with three to four rings, however the homologue profile differed between the unburnt and burnt soils. The PAHs' composition profile and the isomeric diagnostic ratios indicates that PAHs in forest soils were mainly originated from biomass burning.

Ashes and soils from eucalypt hillslopes were consistently enriched in Hg relatively to pine areas, and the opposite was true for Co, Ni and PAHs levels.

Time-since-fire has shown to influence metals and PAHs in three different ways: concentrations of Mn, Cd and PAHs declined abruptly after the first rainfall event (four months after the fire); concentrations of V, Ni and Co increased during the first eight months after the fire; and levels of Cu, Hg and Pb hardly changed during the study period. Additionally, ashes also revealed peak concentrations of metals and PAHs immediately after the fire, with a sharply decline four months later. These decreases could be related to the heavy rainfall and subsequent overland flow that have promoted the washout and leaching of these elements from soils and ashes.

The total PAHs loads in the runoff samples were markedly higher (four times) following the first rainfall event after the wildfire than one year later and also had noticeably different compositions in terms of ring-based as well as individuals PAHs. Both runoff samples was found to induce significant decreases in the growth of the two primary producers- *Pseudokirchneriella subcapitata* and *Lemna minor*- and inhibited the luminescence of the bacteria *Vibrio fischeri*. Conversely, the invertebrate - *Daphnia magna*, was not significant affected. Surprisingly, the runoff collected one year after the wildfire has shown to be the most toxic to the tested species.

Overall, findings of this study brings a new insight on the role of wildfire and subsequent rainfall in the mobilisation of metals and PAHs in ashes and soils from burnt forest plantations, which constitutes an important source of diffuse pollution. In addition, this study also emphasize the risks posed by these contaminants to downstream water bodies, with deleterious consequences for both chemical and biological conditions.

In view of that, this study may be a starting point for design and implement future monitoring programs in burnt areas as part of post-fire management plans.

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Chapter I

Introduction

1. Introduction

1.1 Wildfires in the Mediterranean region

The Mediterranean region is one of the world's biodiversity hotspots due to its outstanding high plant species richness and endemism (Myers et al., 2000; Médail and Diadema, 2009). Mediterranean forests are generally characterized by a remarkable set of features (multiple goods and environmental services, such as preservation of soil and water resources, protection against erosion and land degradation, carbon sequestration and biodiversity conservation) and an exceptionally large variation of environmental conditions (FAO, 2013). Despite the fact that forest have been strongly linked to human activities for several centuries, the current anthropogenic pressure has been negatively impacted the environment (Pausas et al., 2008), particularly as a result of the urbanization, population growth, rural exodus, the overexploitation of natural resources, overgrazing, abandonment of traditional agricultures practices, climate changes and increasing risk of forest fires. The effects of these pressures are responsible for the forest degradation in many places, compromising their essential ecosystems services (Pausas and Keeley, 2009; FAO, 2013).

Forest fires are a natural earth system processes that have occurred for millennia, playing a large role in shaping ecology and evolution of Mediterranean landscape, vegetation and soils (Schmidt and Noack, 2000; Bond and Keeley, 2005; Guénon et al., 2013). Most of Mediterranean forests are adapted to frequent and low-intensity wildfires caused by natural events (spontaneous fires). Moreover, in controlled conditions (prescribed fires), fires have been applied in the management of Mediterranean ecosystems (Pausas and Keeley, 2009; FAO, 2013). In fact, they can control understory vegetation growth, limit fuels loads, prevent severe wildfires (which can have a huge impact on ecosystem functions and resilience) (FAO, 2013), increase the nutrients availability for plants (Neary et al., 1999), and play a key role on the recovery and conservation of certain habitats (Bisson et al., 2003). However, unlike what happened in the past, present fire regimes are not driven by natural or controlled processes, (Pausas et al., 2008; Pausas and Keeley, 2009).

Wildfires are a frequent phenomenon in Mediterranean ecosystems (Granged et al., 2011a, 2011b; Shakesby, 2011) and are considered one of the major causes of soil erosion, land degradation and desertification (Shakesby and Doerr, 2006; Shakesby, 2011) causing serious environmental and economical impacts (Lavorel et al., 1998). In

Portugal, as well as in other Mediterranean countries of Southern-Europe (France, Greece, Italy and Spain), the number of wildfires and burnt area has been impressive over the past decades (Figure 1; Pausas, 2004; Shakesby, 2011; European Commission, 2015).

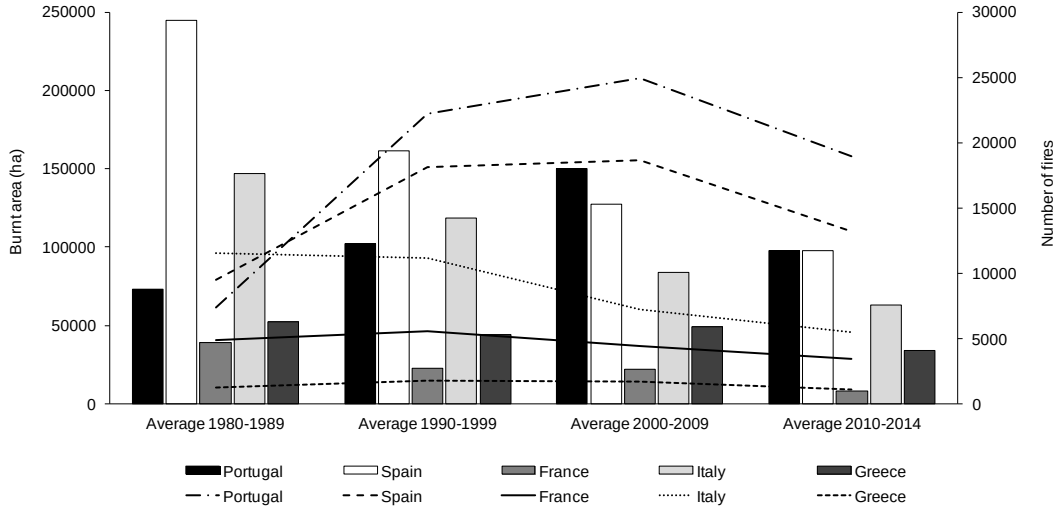

Figure 1 – Average burnt area (ha) and number of fires in five Mediterranean countries of Southern-Europe in the last decades [(1980-2014); European Commission, 2015).

In the Portuguese case, wildfires have consumed an average of 130 000 ha per year in the last 15 years and over 400 000 ha in a dramatic year such as 2003 (Figure 2; ICNF, 2015).

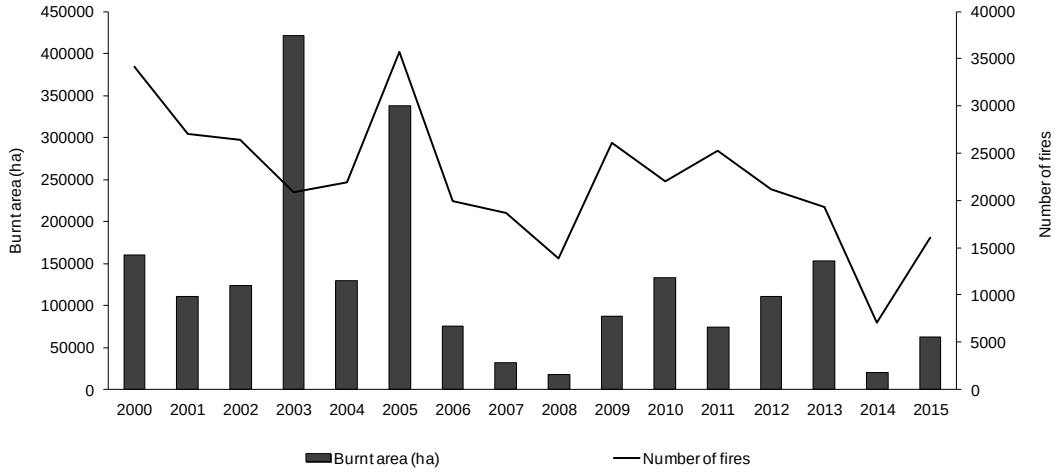

Figure 2 – Burnt area (ha) and number of wildfires occurrences in Portugal in the last 15 years (ICNF, 2015).

Wildfires are more prone to occur in summer season, characterized by high temperatures, low relative air humidity and high availability of fuel mass (Pausas, 2004; Trigo et al., 2006; Carvalho et al., 2008, 2010). In the future, forest fires are likely to become increasingly frequent and severe, not just because of the foreseen increase in fire-propitious meteorological conditions (e.g., longer droughts and heat waves) due to climate change (IPCC, 2013) but also due to underlying causes, which are in part societal, reflecting rural depopulation, land abandonment and afforestation with fire-prone species like pine (e.g. *Pinus pinaster*) and eucalypt (e.g. *Eucalyptus globulus*) (Moreira et al., 2009, 2011; Carmo et al., 2011). Furthermore, there are other anthropogenic causes contributing to this trend, mainly criminal behaviour and negligence (Pereira et al., 2005). The consequences of the intensification of wildfires in the Mediterranean forests are particularly severe, reducing their capacity to naturally regenerate and leading to soil degradation, water scarcity and biodiversity losses (Shakesby, 2011; FAO, 2013).

It is well known that wildfires events are responsible for adverse and diversified impacts on atmospheric, terrestrial, and aquatic environmental compartments, with economic damages and costs and social repercussions (e.g., Smith et al., 2011; Verma and Jayakumar, 2012; Liu et al., 2014). The comprehension of the ecological role of fire is based in the deep knowledge of the forest origin, structure and evolution and in the complex interaction of forest elements such as vegetation, fauna, soil biota, soil properties, topography and climate, among others, which makes the forest a live and dynamic systems (Macedo, 1993). Each ecosystems shows a particular response to fire: some have a high capacity to develop mechanisms that turn them more resilient to fire and others are more fire sensitive (Castro et al., 2012). Several studies regarding the effects of wildfires in the terrestrial compartment, reveal post-fire hydrological and geomorphological effects as well as changes in soil properties and vegetation (Neary et al., 1999; Moreira et al., 2003; Coelho et al., 2004; González-Pérez et al., 2004; Certini, 2005; Shakesby and Doerr, 2006; Malvar et al., 2011; Shakesby, 2011; Maia et al., 2012; Moody, 2013). In the extensive research that exists on the impacts of wildfires on ecosystems there is a growing interest on the effects of fire on soil properties.

1.2 The impacts of wildfires on soil

Soil is a natural element consisting of both mineral and organic components in addition to physical, chemical and biological properties. Although the composition of soil is extremely diverse and governed by many different abiotic and biotic factors, it is

composed of three phases: (1) solid [mineral (46%) and organic (4%)]; (2) liquid (25%) and (3) gaseous (25%). The soil exhibits properties resulting from the physical and chemical equilibrium of these three elements, as well as by its mineral structure and the state of dispersion. Although trace elements are minor components of the solid soil phase, they play an important role in soil fertility (Kabata-Pendias and Mukherjee, 2007).

Soil is a vital non-renewable resource that is not only a component of the ecosystem but also plays a central role for humans, as their survival is tied to soil productivity (Kabata-Pendias and Mukherjee, 2007; Morvan et al., 2008). Moreover, soil provides numerous ecosystem services (regulation, habitat, production and information functions; De Groot et al., 2002), which are of crucial importance for human life and society via processes and products such as nutrient cycling, climate regulation, soil retention, water filtering, buffering, storage and transfer, food security, aesthetic information, degradation of organic contaminants, and protection against the effects of trace element pollution, among others (De Groot et al., 2002; Kabata-Pendias and Mukherjee, 2007). Therefore, it's of crucial importance for the ecosystems and society to assure the protection, quality and sustainability of soil (Morvan et al., 2008; Swartjes et al., 2012).

Forest ecosystems undergo recurrent wildfires with severe impacts resulting from the destruction of vegetation and litter coverage together with severe alterations in the soil properties (e.g. Cerdà and Lasanta, 2005; Neary et al., 2005; Verma and Jayakumar, 2012; Rubenacker et al., 2012). All these changes can compromise and impact the aforementioned soil functions (Doerr and Cerdà, 2005; Verma and Jayakumar, 2012, Guénon et al., 2013). Hence, viewed as an important environmental disturbance, distinct fire related topics have been investigated in order to understand the short-to-long term effects of wildfires on soil functioning (e.g. Certini, 2005; Doerr and Cerdà, 2005; Shakesby and Doerr, 2006; Shakesby, 2011; Verma and Jayakumar, 2012, Guénon et al., 2013).

1.2.1 Wildfire effects on soil properties

At the soil level, the wildfire impacts can be divided into two groups (Neary et al., 1999): (1) direct, as a result of the temperatures reached in the soil, leading to the combustion of organic matter (with consequent reduction in structural complexity and increase in pH and electric conductivity due to the incorporation of soluble cations, such as, carbonates, bases and oxide); (2) indirect, resulting from changes in other components of the ecosystems, such as, reduced vegetation cover, charred litter, or

deposition of partially burnt plant residues and ash (Shakesby and Doerr, 2006; Cerdà and Doerr, 2008) and changes on microbial fauna activity (Shakesby and Doerr, 2006). In turn, these direct and indirect impacts lead to post-fire hydrological (e.g. decreased infiltration and increased overland flow) and geomorphological effects (e.g. changes in sediment transport dynamics of the burnt areas and higher vulnerability to erosive processes) (Shakesby and Doerr, 2006).

The impacts of wildfires can have profound and diversified effects on soil, either physically (e.g. water repellence, particle size distribution, bulk density, aggregate stability, colour and runoff response), chemically [e.g. availability of nutrients, not only nitrogen (N) and phosphorous (P), but also others macronutrients like sodium (Na), magnesium (Mg), sulphur (S), potassium (K), and calcium (Ca) as well as micronutrients like boron (B), iron (Fe), zinc (Zn) and molybdenum (Mo), quantity and quality of organic matter, cation exchange capacity, pH, electric conductivity and production and/or mobilization of contaminants [major and trace elements (metals), polycyclic aromatic hydrocarbons and dioxins] or even biologically (e.g. biomass and composition of microbial and soil-dwelling invertebrates communities and carbon sequestration). These impacts can affect the sustained productivity of soil and its post-fire recovery (González-Pérez et al., 2004; Certini, 2005; Doerr and Cerdà, 2005; Terefe et al., 2008; Verma and Jayakumar, 2012, Guénon et al., 2013). The magnitude of the effects of fire on soil properties depends mainly on the nature of the soil, type and biomass of the vegetation coverage, fire severity, ash production, and post-fire climate conditions, such as timing and intensity of precipitation events (e.g. Ulery et al., 1993, Neary et al., 1999; Certini, 2005; Maia et al., 2012; Verma and Jayakumar, 2012). All the effects of fire on soil have implications on the hydrological cycle due to changes in porosity, infiltration and storage capacity. Furthermore, an important soil property particularly affected by fire and with huge implications in the post-fire hydrological response is the induced or enhanced soil water repellency through the formation of a hydrophobic layer that results in increased runoff and soil losses (Shakesby and Doerr, 2006; Jung et al., 2009; Shakesby, 2011).

One of the most intuitive and important soil change occurred during burning is the alteration of organic matter content (Certini, 2005). Organic matter is a key factor for forest soil since it has a direct and/or indirect influences on the physical, chemical and biological characteristics of the soil. Fire affects soil organic matter both quantitatively and qualitatively and its changes may range from its almost total destruction to increased contents in surface layers due to external inputs, as partly charred litter and leaves, forest necromass and residual ash (González-Pérez et al., 2004; Knicker et al., 2005; Certini, 2005; Mataix-Solera et al., 2011; Faria et al., 2015).

Ash properties and its effects on soil

Another important consequence of forest fire is the deposition of a more or less dense layer of ash on the topsoil (Mandal and Sengupta, 2006), composed of mineral and charred organic components, including charcoal, as a consequence of biomass combustion processes (Bodí et al., 2014). These fire residues are mainly in the form of oxides, hydroxides and carbonates and the major inorganic components of ash are magnesium (Mg), silicon (Si), potassium (K) and calcium (Ca), and, to a lower extent by sodium (Na), phosphorous (P), sulphur (S), and by other major and trace elements (that will also be termed as metals), such as aluminium (Al), vanadium (V), chromium (Cr); manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), cooper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg) and lead (Pb) (Someshwar, 1996; Demeyer et al., 2001; Engle et al., 2006; Pitman, 2006; Plumlee et al, 2007; Pereira and Úbeda, 2010; Gabet and Bookter, 2011; Viana et al., 2012; Bodi et al., 2014). Furthermore, wood ash may also contain some other organic compounds, like polycyclic aromatic hydrocarbons (PAHs) and dioxins in quantified concentrations (Someshwar, 1996; Kim et al., 2003, 2011; Silva et al., 2015). Ash is also characterized by a high alkalinity (Etiegni and Campbell, 1991; Khanna et al., 1994).

Ash produced by wildfires is a very heterogeneous material and the amount of ash deposited, its spatial distribution and their physical and chemical properties depend, among other factors, on the plant species, type and part burnt, fire temperature, combustion completeness and soil type (Demeyer et al., 2001; Goforth et al., 2005; Gabet and Bookter, 2011; Balfour and Woods, 2013; Bodí et al., 2014). The colour lightness of ash can indicate whether combustion of organic matter is incomplete or more through (Goforth et al., 2005; Bodí et al., 2011) and on that basis, ash colour has been used as an indicator of fire severity (Keeley, 2009). Incomplete combustion, which typically occurs at low-medium temperature/severity fires (below 450 °C) and in the absence of oxygen, results in organic-rich ash, mainly in the form of pyrogenic carbon (C), with low density and dark coloured (Masiello, 2004; Goforth et al., 2005; Forbes et al., 2006; Santín et al., 2012; Bodí et al., 2014). This ash resulted from incomplete combustion also presents other elements in smaller proportions like organic nitrogen (Goforth et al., 2005; Knicker, 2011) and concerning contaminants, such as polycyclic aromatic hydrocarbons (PAHs) (Someshwar, 1996; Kim et al., 2003, 2011; Silva et al., 2015). At higher temperature/severity (above 450 °C), oxidation is more intense (provided that sufficient oxygen is available), organic compounds are almost completely combusted and ash colour becomes lighter (grey and white), with high density and

mainly composed of inorganic compounds (Ulery et al., 1993; Goforth et al., 2005; Úbeda et al., 2009; Pereira et al., 2012; Balfour and Woods, 2013; Bodí et al., 2014).

The presence of an ash layer on a burnt site is often short-lived with ash being rapidly mobilised both by rainfall and wind with its soluble elements being dissolved during rainfall and transported by overland flow to water bodies, or incorporated into the soil (Bodí et al., 2011; Santín et al., 2015). However, the presence of ash on the soil surface in the initial period after fire can have huge impacts on terrestrial and aquatic ecosystems (Bodí et al., 2011; Bodí et al., 2014; Santín et al., 2015). It affects the soil microbial community, plant germination and the fertilization effect on plant growth (Demeyer et al., 2001; Pitman, 2006; Bodí et al., 2014). In general, when ash is incorporated in the soil, it stimulates microbial activity due to the amelioration of soil physical and chemical characteristics (Raison, 1979; Demeyer et al., 2001; Mataix-Solera et al., 2011). Regarding plant germination, the ash layer can inhibit plant germination by preventing water from reaching the seed given the high osmotic pressure or due to the toxic effects of some ions or alkaline pH (Ne'eman et al., 1993; Bodi et al., 2014). In contrast, field and greenhouse experiments confirmed that plants benefit in their growth from the nutrients (N and P) and Mg^{2+}, K^{+} and Ca^{2+} availability contained in wood ash and the increase in microbial activity (Raison, 1979; Chambers and Attiwill, 1994; Demeyer et al., 2001; Bodí et al., 2014).

Ash incorporated into the soil can have effects on the physical and chemical properties of the soil, as for example, it increases temporarily soil pH and electric conductivity (Demeyer et al., 2001; Badía and Marti, 2003; Certini, 2005; Schafer and Mack, 2010) and is a significant source of nutrients (N and P) for the soil (Khanna et al., 1994; Demeyer et al., 2001; Badía and Marti, 2003; Bodí et al., 2014), especially in the immediate period after the fire, when the majority of the nutrients are leached (Cerdà and Lasanta, 2005; Lasanta and Cerdà, 2005). Ash produced at low-medium temperature/severity can increased the soil organic matter by the incorporation of pyrogenic C into the soil through biological and physical mechanisms (Eckmeier et al. 2010; Bodí et al., 2014). As a consequence of this pyrogenic C input from ash, the soil organic matter may change its quality (for example, increasing its aromaticity) (Bodí et al., 2014). Substantial increases in some macroelements, especially Mg, K and Ca in soil after ash deposition, have been reported (Demeyer et al., 2001; Pereira et al., 2014) and with lower increases of Na and Si, (Khanna et al., 2004; Bodí et al., 2014). In terms of major and trace elements, such as Al, V, Mn, Fe, Ni, Cu, Zn, Cd, Pb, ash can have a small contribution for their increases in soil (Parra et al., 1996; Mandal and Sengupta,

2006; Zhai et al., 2009). The same is true for the PAHs amounts in soils (Someshwar, 1996; Kim et al., 2003, 2011).

Ash can also have important effects in the hydrological behaviour and hence in soil erosion processes (Bodí et al., 2011; Badía and Marti, 2003; Cerdà and Doerr, 2005, 2008; Onda et al., 2008; Woods and Balfour, 2010; Balfour et al., 2014). Ash modifies soil hydrologic behaviour by creating a two-layer system: the soil and the ash layer, which can function in different ways depending on (1) the variability of ash physical and mineralogical properties (e.g. ash water holding capacity, hydraulic conductivity, potential to clog soil pores and consequently limiting infiltrations rates, ash wettability vs. ash water repellency) at different combustion conditions (Etiegni et Campbell, 1991; Cerdà and Doerr, 2008; Stoof et al., 2010; Woods and Balfour, 2010; Bodí et al., 2011; Balfour et al., 2014; Bodí et al., 2014); (2) the thickness of ash layer (Cerdà and Doerr, 2008; Woods and Balfour, 2010; Bodí et al., 2012; Bodí et al., 2014); (3) soil type [e.g. particle size, porosity (Larsen et al., 2009; Woods and Balfour, 2010) and (4) rainfall characteristics (timing, duration and intensity; Bodí et al., 2014). However, the results of all these studies suggest a variable response of the effects of ash on soil hydrological processes. This way, the hydrological responses are not straightforward and ash impacts on runoff and transport processes can be contradictory: in some cases ash can increase the overland flow rates (Onda et al., 2008; Woods and Balfour, 2010) and in other cases the opposite was reported (Cerdà and Doerr, 2008; Woods and Balfour, 2008; Bodí et al., 2012).

It has been shown that wildfires have the ability to produce and mobilize contaminants, and therefore fire ashes are rich in several concerning elements. For example, combustion of plants and natural materials has been reported to release metals (Caldwell et al., 2000; Amirbahman et al., 2004; Burke et al., 2010; Santín et al., 2015), polycyclic aromatic hydrocarbons (PAHs; Radojevic, 2003; Ravindra et al., 2008; Kim et al., 2003), dioxins (Gullet and Touati, 2003; Meyer et al., 2004) and nitrogen and phosphorous compounds (Meixner et al., 2006; Jung et al., 2009; Smith et al., 2011; Ferreira et al., 2016), which are subsequently mobilized.

1.2.2 Wildfire effects on metals mobilisation

Soil perform vital functions, which support plant, animal and human life. In particular, soil is not only an important sink for major and trace elements, but it's also one of the most important environmental compartments in the biogeochemical cycle of metals, acting as a natural buffer controlling the transport of major and trace elements (metals) to the atmosphere, hydrosphere, and biota (Kabata-Pendias and Mukherjee, 2007; Kabata-Pendias, 2011). In addition soil is the primary source of metals for plants, animals and humans (Hooda, 2010). Notwithstanding the importance of some metals as essential elements for growth, development and biological life, they become toxic for life depending on the doses. Among the distinct metals, some are of particular environmental concern due to their high toxicity, environmental persistence and tendency to bioaccumulate in the food chain (Adriano, 2001; Ignatavièius et al., 2006). Metals of major interest given the risk they represent to organism (Be, Cr, Ni, Cu, Zn, As, Se, Ag, Cd, Sb, Hg, Ti and Pb) are included in the USEPA list of priority pollutants (USEPA, 2014). As for the European Commission, Ni, Cd, Hg and Pb are the ones included on the Priority Substances Directive (European Commission, 2008). Vanadium, Mn and Co are also of major interest in bioavailability studies (McKinney and Rogers, 1992). Likewise, Hg is of particular concern due to its volatility and relevance of the atmospheric compartment in the global Hg cycle (Cheng and Schroeder, 2000; Huang et al., 2011). Once deposited, Hg undergo methylation by aerobic and anaerobic bacteria into methyl mercury species that are highly toxic, mobile and bioavailable with a strong capacity to bioaccumulate and then biomagnify along the food chain (WHO, 1991, 2003; Boening, 2000; Amirbahman et al., 2004).

In soil, metals are distributed over the different soil compartments (Figure 3) where they exist in a variety of chemical association forms, strongly differing in reactivity, which are highly element specific. These forms and their subsequent transformations are determined by the dynamic characteristics of a specific soil environment, which is defined by the composition of the soil, biological activity, water flows, and temperature.

Metals in the soil fraction vary in degree of mobility. Their bioavailability is regulated by physical, chemical and biological processes and the interactions between them. Hence their bioavailability depends on several soils characteristics, which includes: (1) pH and Eh values; (2) cation exchange capacity; (3) granulometric composition; (4) organic matter; (5) oxides and hydroxide, mainly Fe, Mn and Al; (6) activity of microorganisms; (7) occurrence and forms of cations. This way, metals associations with the particular soil phase and soil component appear to be fundamental in defining their

behaviour (Kabata-Pendias, 2011). All of these factors also determine how many metallic elements will be accumulated in biological material, how many will be subjected to the process of adsorption, complexation or will be immobilized in the soil particles. Knowledge on the association of metals with particular soil phases and their affinity to each soil constituent is the key to a better understanding of the principles governing their behaviour in soils (Kabata-Pendias and Mukherjee, 2007; Kabata-Pendias, 2011). The behaviour of each element in soils is very complex. Its environmental and toxicological effects are however, highly depended on the element forms (different states of oxidation), as different species interact differently with soil constituents, exhibiting different behaviours, solubility, mobility, bioavailability and toxicity.

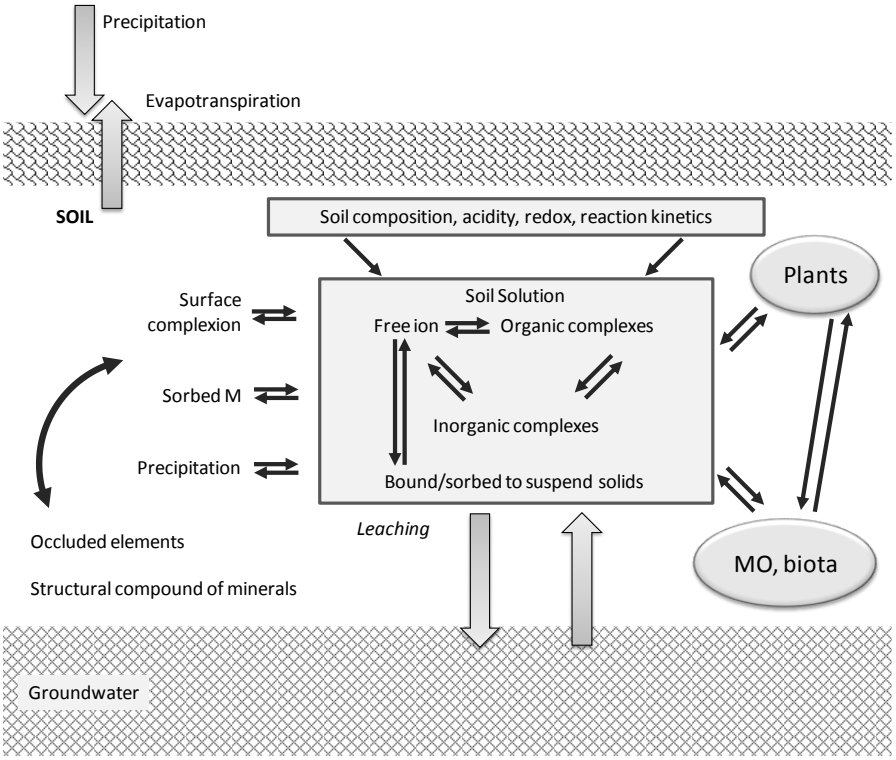

Figure 3 – Schematic representation of various pools of metals in the soil system. The soil solution is the central gateway through which forms of elements interacts with the solid phase and soil biological activity (Adapted from Hooda, 2010).

Accumulation of metals in forest soils has been found provoke toxic responses in soils microorganisms (Certini, 2005; Hart et al., 2005), including by reducing microbial biomass (Hartmann et al., 2005) and by decreasing carbon mineralization and disturbing enzymatic activities (Frey et al., 2006). Metal stress has furthermore been found to change the structure and diversity of microbial communities (Hartmann et al., 2005; Frey et al., 2006).

Metals can occur naturally in the soil environment from various origins: lithogenic (directly inherited from the lithosphere- parent material) and pedogenic (from lithogenic origin also, but their concentration and distribution in soil layers and particles are changed due to soil-forming processes- pedogenic processes). However, metals can also be introduced into soil through a variety of anthropogenic sources (as a result of human's activities), including atmospheric deposition, metal-smelting industries, coal and fuel combustion, metal-containing fertilizers or pesticides, transport emissions, sewage sludge and others (Adriano, 2001; Alloway, 1995). Although generally neglected, wildfires constitute an additional diffuse source of metals to the soils. In fact, wildfires have been found to release and deposit metals on the soil surface, either directly by combustion of vegetation and mineralization of soil organic matter or indirectly through interactions of ashes with the underlying-soil (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014). Both processes can lead to the addition of potentially significant amounts of these elements in the soil and, thereby, can alter its chemical properties (Ulery et al., 1993; Antilén et al., 2006; Jakubus et al., 2010; Pereira and Úbeda 2010). Diffuse inputs of metals into the environment through, for example, forest fires are also an environmental challenge because of their tendency for accumulation over time, with long lasting effects after elimination of their major sources (Chen et al., 1997; Sun et al., 2001; Bretzel and Calderisi, 2006; Hjortenkrans et al., 2006). Hence, among the effects of wildfires, it is important to recognize and underline its impact on the chemical availability of metals and their pathways (Navrátil et al., 2009; Jakubus et al., 2010; Aref et al., 2011).

Mobilization of metals by lateral transport, either in dissolved or particulate form, is likely to be significant in recently burnt areas, as fire typically enhances overland flow generation and the associated transport of ash and soil particles to downstream water bodies during periods that can last several years (Cannon et al., 2001; Lasanta and Cerdà, 2005; Malvar et al., 2011; Moody et al., 2013; Prats et al., 2014). These post-fire pollutant fluxes will vary depending on a variety of factors such as fire intensity, vegetation composition and plant parts burnt (i.e. leaves, bark, twigs, litter), soil type, meteorological conditions before after and during fire, and post-fire hydrological and erosion response (Ulery et al, 1993; Someshwar, 1996; Werkelin et al., 2005; Wang and Dibdiakova, 2014; Machado et al., 2015). Albeit fire has an important role on the mobilisation of trace metals, this have been a relatively neglected issue.

Fire-induced increases in metals in soils concentrations have been reported by several authors (Parra et al., 1996; Plumlee et al., 2007; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014). Manganese concentrations in forest soil has been found

to increase as a result of the fire, through additions of Mn attached to ash and the physicochemical breakdown of Mn complexed with organic matter (Auclair, 1977; Chambers and Attiwill, 1994; Parra et al., 1996; Costa et al., 2014). Furthermore, Costa et al. (2014) reported enrichment Mn in ashes relatively to the underlying soils up to 5 times. Plumlee et al. (2007) reported significant increased influence of southern California wildfires in the concentration of V, Mn, Co, Ni, Cu, Cd and Pb in burnt soils (Plumlee et al., 2007). Bogacz et al. (2011) also found pools of Ni, Cu and Pb in soils collected immediately and 2 years after a fire, comparatively to soils collected 12 and 21 years after a fire, and Jovanovic et al. (2011) showed that post-fire area had increased content of Cu and Pb. Conversely, Costa et al. (2014) observed that the wildfire did not influence the concentrations of Cu in soils from burnt areas. Slightly elevated concentrations of Cd were also observed in burnt soils (Jovanovic et al., 2011). Surface soils polluted with fly ashes showed V contents up to 429 mg kg^{-1} (Morrell et al., 1986). Mandal and Sengupta (2006) observed enrichments of Co in soils collected near ash ponds.

In general, the major and trace elements (metals) above mentioned showed an important enrichment in surface burnt soils, which can be derived from the ash deposition after the wildfire, which are very rich in these elements (Mandal and Sengupta, 2006; Zhai et al., 2009). This way, metals are probably adsorbed into the ash particles, then finally deposited and mobilized onto the soil surface, suggesting that their concentration in soil is strongly influenced by the deposition of ashes resulting from the combustion of wood a litter.

Recent studies point out that the volatilization of Hg (from vegetation, litter and soils) during wildfires is an important pathway for the re-emission of atmospherically deposited Hg (Friedli et al., 2001, 2003; Sigler et al., 2003; Engle et al., 2006). The amount of Hg released during a wildfire is limited by Hg accumulation in the ecosystem, particularly in the soil, prior to burning (Biswas et al., 2007, 2008). Large Hg losses result from the low volatilization temperatures (100-300°C) of Hg species. Experimental burns performed on soils showed a 79% decrease in Hg content after burning (Mailman and Bodaly, 2005). Recent studies have reported Hg release from soils burned during both prescribe fires (Woodruff et al., 2001; Harden et al., 2004; Engle et al., 2006) and wildfires (Biswas et al., 2007, 2008; Engle et al., 2006; Navrátil et al., 2009; Burke et al., 2010).

The observed changes in soil chemical properties could affect soil functioning through their impacts on the composition and activity of microbial communities. Overall, increased wildfire frequency combined with intensified post-fire rainfall are viewed as

important threats to soil productivity and ecosystem resilience in the long-term, through their implications not just on soil (fertility) losses by erosion but also on the decline in microbial mineralising activities (Hernández et al., 1997; Pardini et al., 2004; Guénon et al., 2013; Goberna et al., 2012; Muñoz-Rojas et al., 2016).

Due to the persistence of metals in the environment, long-term studies are critical to better understand the delayed and persistent effects of fire on metal mobilization in forest soils (Miesel et al., 2012).

1.2.3 Wildfire effects on PAHs production and mobilisation

Polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants, which have caused major concerns in terms of public health and environment due to their toxic, carcinogenic, mutagenic and teratogenic properties (IARC, 1991, 2010; ATSDR, 1995; European Commission, 2006; Wang et al., 2013). Additionally, such hydrophobic compounds rapidly adsorb onto particles, and show a high environmental persistence and bioaccumulation potential (IARC, 1998; ATSDR, 1995; Delistray, 1997; Boström, 2002; Rappaport et al., 2004; Cachot et al., 2006). For this reasons, several international organizations [World Health Organization (WHO), US environmental Protection Agency (USEPA), Agency of Toxic Substances and Disease Register (ATSDR), International Agency for Research on Cancer (IARC) and European Community (EC)] have classified some PAHs among the most hazardous and persistent organic pollutants (POPs), being listed as priority pollutant. The USEPA has placed fifteen PAHs compounds on the priority pollutant list: naphthalene (NAP) (two-ring compound) acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT) (three-ring compounds); fluoranthene (FLT), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR) (four-rings); benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(ah)anthracene (DBahA), (five-rings); indeno(1,2,3-cd)pyrene (IND) and benzo(g,h,i)perylene (BghiP) (six-rings) (USEPA, 2014). The NAP, ANT, FLT, BbF, BkF, BaP, IND and BghiP are also included in the list of priority substances (European Commission, 2008). Furthermore, five of these PAHs are considered to be possible or probable carcinogens (ATSDR, 1995)

Polycyclic aromatic hydrocarbons (PAHs) comprise a group of organic compounds made up of carbon and hydrogen atoms grouped into two or more condensed aromatic ring structures in a linear, angular, or clustered arrangement. However, alkyl-substituted PAHs, heterocyclic PAHs containing nitrogen, sulphur and oxygen, and oxidation

products of PAHs (oxy-PAHs) – including PAH ketones, PAH quinines, and hydroxylated PAHs – are often grouped together with unsubstituted PAHs and are often referred as polycyclic aromatic compounds (PACs) (Harvey, 1997; Fetzer, 2000).

The PAHs compounds are usually divided into two categories: low molecular weight compounds (LMW) with two or three rings and high weight compounds (HMW) with more than three rings. Furthermore, PAHs are also classified as alternant and nonalternant based on ring structure. Alternant PAHs are derived from benzene by fusion of additional six-membered benzoid rings, while nonalternant PAHs containing at least one five-member ring in addition to the six-membered rings (Harvey, 1997).

The physicochemical properties of PAHs, which vary with their molecular weight and structure, largely determine their environmental behavior. Except for naphthalene, PAHs have very low to low water solubility and low to moderately high vapour pressures. Their octanol-water partition coefficients (reflecting the hydrophobicity) are relatively high, indicating a relatively high potential for adsorption to suspended particulates in the air and in the water, and for bioconcentration in organism (IARC, 1998; Pierzynski et al., 2000; Lundstedt et al., 2003; Cachot et al., 2006). As molecular weight increases, hydrophobicity/lipophilicity increases, water solubility and vapour pressure decreases, and the compounds will have a more recalcitrant (difficult to degrade) structure. Therefore, LMW PAHs are more soluble and volatile (being susceptible to atmospheric degradation processes), and hence more mobile and available than HMW. The generally higher availability of LMW PAHs, as compared to HMW PAHs, make them more susceptible to various biological, chemical and photochemical degradation processes. The HMW PAHS, are more persist in the environment because of their low volatility, resistance to leaching and recalcitrant nature (Wild and Jones et al., 1995; Harvey, 1997; Lundstedt et al., 2003), indicating that transfer and turnover will be more rapid for LMW PAHs than for the HMW PAHs (Lundstedt et al., 2003).

Polycyclic aromatic hydrocarbons are formed and released into the environment through natural or anthropogenic sources, usually occurring as complex mixtures. The prevailing emissions are caused by human activities, mainly by the incomplete combustion of fossil fuels (coal, oil and natural gas) and wood burning. Other anthropogenic sources include fuel an oil splits, natural seeps, domestic emissions (home eating, cooking, etc.), agricultural (burning of biomass), mobile (emission from vehicles, shipping, railways, automobiles, off-road vehicles and machinery) and industrial sources (e.g. coke production, waste incineration, cement manufacture, petrochemical, and related industries) (Lundstedt et al., 2003; Marchand et al., 2004; Ravindra et al., 2008). In nature, PAHs may be formed in three ways: (1) pyrogenic PAHs are produced

from high-temperatures pyrolysis of organic materials- forest fires and volcanic eruptions; (2) petrogenic PAHs come from low to moderate temperature diagenesis of sedimentary organic material to form fossil fuels; and (3) biogenic PAHs are originated from direct biosynthesis by microbes and plants (Neff, 1979; Ravindra et al., 2008).

Polycyclic aromatic hydrocarbons are ubiquitous and found in air, water, terrestrial and biological systems (Bjorseth et al., 1979; Gao and Zhou, 2004). The global movement of PAHs is summarized in Figure 4 (Pierzynski et al., 2000; Yu, 2005). PAHs emitted into the atmosphere [mostly found adsorbed to particulate matter, but although also found in the gaseous phase; (NRC 1983; Yang et al., 1991)] can be transported through the air over short and long distances but are eventually deposited in terrestrial and aquatic environments, through dry or wet deposition (Van Jaarsveld et al., 1997; Ding et al., 2007; Choi et al., 2009). PAHs can be deposited directly onto the soil but can also end up into the soil through deposition on or absorption by plants and, subsequently, wash-off by throughfall and stemflow or litter fall and decomposition (Neff, 1979; Eisler, 1987). Although, PAHs in soils are strongly adsorbed to organic soil particles, they can volatilize (Park et al., 1990; Wild and Jones, 1995), can undergo abiotic degradation (photolysis and oxidation-reductions reactions) and, biodegradation by microbial activity (it's important to refer that the degradation and biodegradation are slowly processes and PAHs can remain in soil for many years), or can be uptake by plants roots or animal ingestion.

Microbial metabolism is the major process for degradation of PAHs in soil environment. Photolysis, hydrolysis, and oxidation generally are not usually considered to be such important processes for the degradation of PAHs in soils (Sims and Overcash, 1983). However, Wild and Jones, 1993, found important losses of LMW PAHs due to volatilization and photolysis in surface soils amended with PAHs, whereas abiotic losses were insignificant for HMW. The environmental fate and behavior of PAHs (including their rate and extent of biodegradation) are also influenced by soil properties, such as organic matter content, texture and aggregation, microbial activity; the presence of contaminants such as metals and cyanides that are toxic to microorganisms; and the physical and chemical properties of the PAHs (Wild and Jones, 1993).

Accumulation of PAHs in soils may lead to contamination of plants (Muller and Shann, 2006), and through the food chain, may cause direct or indirect exposure to humans. Furthermore, PAHs can reach other compartments than just soil, contaminating the atmosphere by volatilization or the aquatic systems by overland flow or leaching (Srogi, 2007). The presence of PAHs in all these systems create a risk not only to human but to all living organisms. In brief, a wide range of ecotoxicological effects in diverse

organisms are described, including microorganisms, terrestrial plants, amphibians, reptiles and mammals (e.g. Wang et al., 1993; Long et al., 1995; Simonich and Hites, 1995; Delistray 1997; Juhasz and Naidu, 2000; Sverdrup et al., 2002; Rappaport et al., 2004; Cachot et al., 2006; Hellou et al., 2006). The reported effects involved lethal and sub-lethal toxicity, affecting survival, growth and/or metabolic activity, as well as developmental and reproductive fitness, enhancing DNA mutation, cancer formation and acting as endocrine disruptors (Delistray 1997; Clemons et al., 1998; Hellou et al., 2006). Knowledge on PAH contents in soils are therefore a prerequisite for avoiding risks to environment ecosystems and human health (Srogi, 2007).

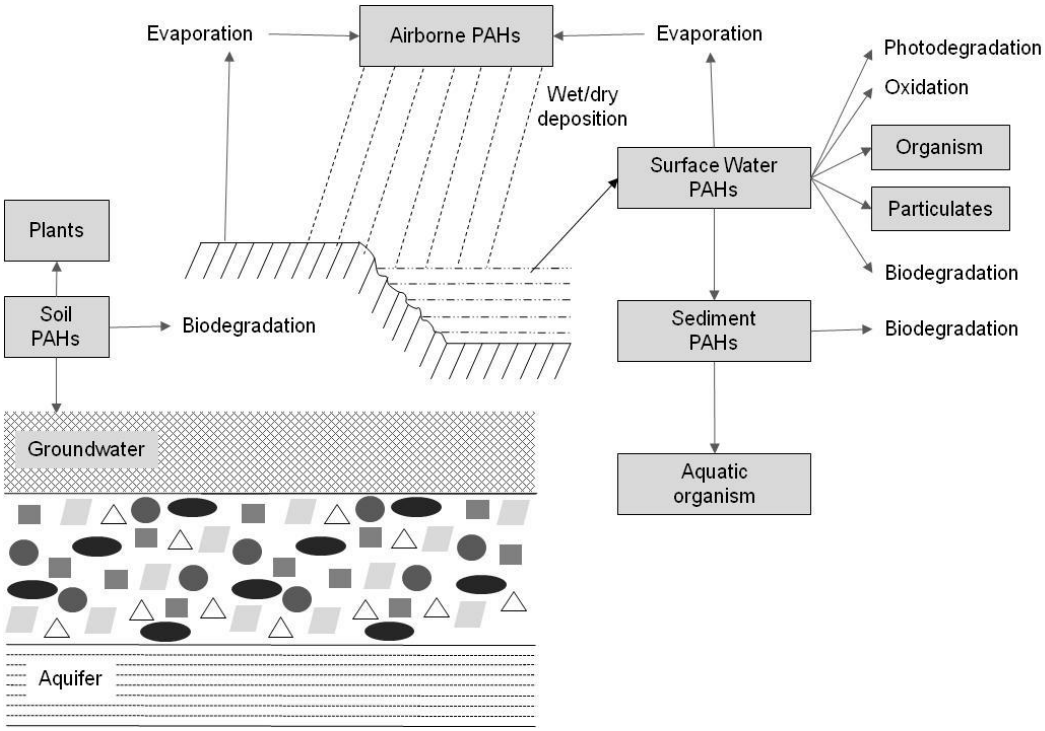

Figure 4 –Transport of polycyclic aromatic hydrocarbons (PAHs) (Adapted from Yu, 2005).

PAH contents in soils and their possible sources have been less well studied for forest soils (Bucheli et al., 2004; Xiao et al., 2014) than for urban and agricultural soils (e.g. Bradley et al., 1994; Tay and Biney, 2013; Wang et al., 2013). Nonetheless, forest soils are widely regarded as an important long-term repository of PAHs, mainly because of the large interception areas of trees and by the high organic matter contents of forest soils (Wilcke et al., 1996; Wilcke, 2000; Srogi, 2007). In turn, apart from the human activities, forest fires have been identified as an important source of airborne PAHs, with smoke from biomass burning revealing high PAH concentrations (Freeman and Cattell, 1990; Baek et al., 1991; Radojevic, 2003; Ravindra et al., 2008). For example, it was

reported that the annual 16 PAHs emission from natural fires of forest and moors in the United Kingdom was 94.9 Mg, of which 2.1 Mg was from accidental forest fires (National Atmospheric Emission Inventory), corresponding to an emission per unit of forest area of $8.5x10^{-5}$ Mg km^{-2} yr^{-1} (UNECE/FAO, 2000). In a study conducted in China to estimate the PAHs emissions from wildfires, Yuan et al. (2008) found that among the 16 PAHs studied, NAP contributed to 55% of the total and the other predominant species were PHE (11%), ACE (7.3%) and ACY (6.9%). The type of biomass burnt and the characteristics of the fire itself such as fire intensity play a key role in the PAHs concentrations of the emitted smoke (Vergnoux et al., 2011). However, the potential contribution of PAHs released into the environment during the wildfire has been less studied, mainly in what concern their contents in forest soils (Kim et al., 2003, 2011; Garcia-Falcón et al., 2006; Vergnoux et al., 2011; Sojinu et al., 2011; Choi, 2014; Tsibart et al., 2014). These former studies suggested that forest fires resulted in elevated levels of PAHs in soil, decreasing with time-since-fire by erosion and leaching of soil and ashes.

High concentrations of PAHs in burnt soils of a mangrove forest in Nigeria and of drained peatland in Russia were reported by Sojinu et al. (2011) and Tsibart et al. (2014), respectively. Kim et al. (2003, 2011) also reported increased levels of PAHs in forest soils (approximately 15 times up the maximums reported for unburnt soils) in Korea 1 month after a wildfire. PAHs are probably adsorbed by ash particles, then finally deposited onto the surface soil, suggesting that the concentration of PAHs in soil were strongly influenced by the ashes (ash-derived PAHs) resulting from the combustion of wood and litter layer (Kim et al., 2003). In addition, burnt soils were characterized by higher levels of two to four rings (NAP and PHE were the dominant compounds) than those of HMW PAHs (Kim et al., 2011). The contribution of forest fires to the increase content of PAHs in burnt soils with the dominance of the two to four rings PAHs (NAP, ACE, FLU, PHE, ANT, FLT and PYR) was also reported by Vergnoux et al. (2011) and Choi (2014), The remarkably higher levels reached by the two to four rings (NAP, PHE, FLU, FLT and PYR) in the burnt soil was in good agreement with the fact that they are the most produced aromatic compounds during wildfires (from wood combustion) rather than BghiP, DBahA and IND (Freeman and Cattell, 1990; Xu et al., 2006; Yuan et al., 2008). Pyrene is also considered, along with PHE and FLT, as one of the major PAHs products from the burning of gymnosperms and angiosperms biomass (Simonelt, 2002). Furthermore, the most volatile PAHs, NAP, FLU, PHE and ANT, as well as the FLT (less volatile than the previous mentioned) appear to be the major PAHs produced by pinewood and needles combustion (Shauer et al., 2001; Conde et al., 2005).

Garcia – Falcón et al. (2006) reported a decreased of 57% in the PAHs levels with time-since-fire in a Atlantic woodland. Similar findings were also observed by Kim et al. (2003, 2011), Vergnoux et al. (2011) and Choi (2014), as well as a reduction in LMW. For example, Vergnoux et al. (2011) reported remarkable reductions one year after the fire for the three-ring PAHs, with 84%, 80%, 76% and 88% removal yields for ACE, FLU, PHE and ANT, respectively. The washout of PAHs associated to soil and ash particles by overland flow (heavy rainfall), their leaching into lower soils layers, the dissipation of ashes by wind, as well as the vaporization and degradation of LMW PAHs, were pointed the main reasons for the observed reductions over time (Kim et al., 2011; Vergnoux et al., 2011; Choi et al., 2014). According to these studies, the levels and patterns of PAHs and their temporal trend in burnt soil can be significantly influenced by the type of vegetation, soil properties and meteorological conditions.

1.3 Wildfires impacts on aquatic systems

It is well document that wildfires through its direct effects on vegetation cover, soil properties, erosion and atmospheric inputs can lead to considerable changes in geomorphological and hydrological processes, as previous discussed (Shakesby, 2011). Among the indirect effects, the generation of fire-induced runoff and soil erosion associated caused by fire and the ash incorporated into the soil surface can impair the sustainability of water bodies (surface and groundwater) within and downstream of the burnt area (Lasanta and Cerdà, 2005; Smith et al., 2011). Previous studies all over the world, including Portugal, have shown that heavy rainfall and the subsequent surface runoff play an important role in soil erosion and therefore on the transport of particles to downstream aquatic systems (Ferreira et al., 2008; Keizer et al., 2008; Malvar et al., 2011; Prats et al., 2014).

A key environmental concern in relation to wildfires is that they constitute a diffuse source of contamination of the aquatic systems affecting the water quality, namely through the production and subsequent mobilisation of deleterious pyrolytic substances, such as PAHs, and also through the input of metals associated to ash/soil loads. We often speak of terrestrial and aquatic ecosystems as though they were separate, but aquatic ecosystems are structured by interactions among terrestrial and aquatic processes and climate.

Overland flow from burnt areas with entrained ash and sediment rich in nutrients (N and P) and other water quality constituents (e.g. C, Na, Mg, K, Ca, metals elements and

PAHs) will temporarily change the chemistry of water bodies, with potentially negative impacts on water quality (Lasanta and Cerdà, 2005; Meixner et al., 2006; Smith et al., 2011; Stein et al., 2012; Machado et al., 2015; Silva et al., 2015). These elements can be leached into soil profile or transported downslope and downstream by runoff impacting groundwater and surface water bodies, respectively (Reneau et al., 2007; Cerdà and Doerr, 2008; Smith et al., 2011) and causing complex changes in community and trophic structure (Rinne, 1996).

It is rather well documented that wildfires can affect the aquatic systems, mainly in terms of water quality constituents, such as, total suspended solids and turbidity (Lane et al., 2006; Wilkinson et al., 2006), nutrients (Burke et al., 2005; Ferreira et al., 2005; Lane et al., 2008; Machado et al., 2015; Ferreira et al., 2016), organic carbon (Minshall et al., 2001a; Wasson et al., 2003; Mast and Clow, 2008), inorganic compounds such as sodium, potassium and calcium (Gallaher et al., 2002; Ferreira et al., 2005; Mast and Clow, 2008; Machado et al., 2015), sulphate (Earl and Blinn, 2003; Mast and Clow, 2008) among others properties.

The influences of wildfires on aquatic systems are not limited to changes in water quality, and they have been shown to impact aquatic biota, including periphyton and phytoplankton (Planas et al., 2000; Charette and Prepas, 2003; Earl and Blinn, 2003; Cowell et al., 2006), macroinvertebrates (Scrimgeour et al., 2001; Minshall et al., 2001a, 2001b; Minshall, 2003; Earl and Blinn, 2003; Mellon et al, 2008, Hall et al., 2008), fish (Rinne, 1996; Spencer et al., 2003) and amphibians (Pilliod et al., 2003).

Notwithstanding almost all of those studies evaluated the impact of the wildfires on ecological properties (density, biomass, composition, taxa richness, etc) in concern of physical and chemical composition of the stream water, including, temperature, pH, conductivity, dissolved oxygen, organic matter, nutrients (nitrogen and phosphorus) and mineral salts (sodium, potassium, lithium, magnesium, calcium, chloride, fluoride, ammonia and sulphate) and/or the characteristics of the ashes.

As aforementioned, besides the well-known effects of wildfires, contamination of water bodies by post-fire inputs of various metals and PAHs may constitute also an environmental problem, as some of them are poisonous at high concentrations and tend to bioaccumulate. Likewise, contamination of water supply reservoirs by PAHs or metals constitute also a concerning risk to human health, either by direct consumption of water with concentrations exceeding threshold values, or by the use of water for recreational activities as fishing and swimming. Thus, the input of metals and PAHs into aquatic systems after forest fires may become a concerning environmental problem, which have been vaguely explored.

The presence of metals (e.g. V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb) in the aquatic systems occur naturally as result of the slow-rate leaching from soil/rock to water or via atmosphere sources (Adriano, 2001; Paliulis, 2006; Zhou et al., 2008). However, wildfires constitute an additional diffuse source of metals to aquatic systems, following increased erosion rates and overland flow that promotes the input of ash and soil in water, as well as the direct input of ash by wind (Ignatavičius et al., 2006; Smith et al., 2011). Metals are of great environmental concern, since they tend to concentrate in aquatic organisms, are virtually non-degradable, and thus produce long lasting effects upon the environment, even after their major sources have been removed. Although, after being released, metals are mostly adsorbed onto suspended particles and finally accumulated in the sediment (which can serve as a source of metals to the overlying water), they are able to be released into the water (in ionic forms) under the suitable conditions, such as pH and redox potential, leading to further contamination of aquatic systems (Miao et al., 2006). Worth mentioning that the sediment-water interaction in aquatic systems play an important role on controlling metals transport processes (Point et al., 2007; Thouzeau et al., 2007).

While a number of metals such as Cu, Zn and Fe are essential to living organisms, for example for protein structure stability, electron transfer reactions and gene regulation (Siegel, 1973), others such as Cd, Pb and Hg may displace or replace essential metals and interfere with the proper functioning of enzymes and associated cofactors, causing noxious effects in aquatic organisms (Siegel, 1973; Gifford et al., 2004). Even the former metals, however, can cause toxic effects if present in elevated concentrations (Alloway, 1995; Sunda and Hutsman, 1998; Gifford et al., 2004). Mercury, after being deposited undergo methylation by biological processes into the highly toxic methyl mercury, a potent neurotoxin with strong tendency to bioaccumulate and then biomagnify within the food chain (Boening, 2000; Gnamus et al., 2000; Amirbahman et al., 2004). Trace elements may accumulate in aquatic organisms through different mechanisms: directly from water, via uptake from suspended particles and sediment, or by the consumption of lower trophic levels organisms (Fernandes et al., 2007; Morrison et al., 2008; Pereira et al., 2016). Therefore, some metals may be transformed into persistent metallic compounds with high toxicity, which can be bioaccumulated in the organisms, magnified in the food chain, thus threatening human health, particularly when it occurs in species of commercial value (Boening, 2000; Gupta and Singh, 2011).

Contamination loading associated with storm water runoff from recently burnt areas is lacking of fundamental understanding, despite the fact that it has the potential to affect downstream water quality. (Plumlee et al., 2007; Hageman et al., 2008; Liodakis et al.,

2009; Costa et al., 2014; Silva et al., 2015; Santín et al., 2015). Stein et al. (2012) reported higher flux and concentrations of Cu, Zn and Pb in runoff from burnt areas compared to unburnt areas after the wildfires occurrence in Southern California, with differences ranging from a 112-fold increase for Cu to a 736-fold increase for Pb. Gallaher and Koch, 2004 also documented Cu, Zn and Pb enrichment in post-fire runoff after the Cerro Grande wildfire in New Mexico. In Marão river (Portugal), Costa et al. (2014) found higher concentrations of Mn in the water systems within the burnt catchment 5, 8 and 12 months after the fire. This increase was probably triggered by the movement of ashes to the watercourses and it persistence was due to the particular redox potential and pH conditions necessary for precipitation of oxides or hydroxides of Mn (Hem, 1898; Costa et al., 2014). Concentrations of water-extractable elements (e.g. Cd, Hg and Pb) in ashes collected following the Balmoral wildfire, Sydney, Australia, were investigated by Santín et al. (2015), that found lower values, ranging from 0.5 to 0.6 μg kg^{-1} for Hg and 3.3 to 13.7 μg kg^{-1} for Pb and Cd was not even detected (0.0 μg kg^{-1}). Silva et al. (2015) in a study conduct in Aveiro, Portugal, in a mixed forest plantation of pine and eucalypt analysed a large group of metals, such as V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb, in ash collected immediately after the fire as well as in aqueous extract of ash (AEA) and reported lower values for some chemical elements. In the AEA, the elements (among the mentioned before) that were above the quantification limits were: V (31 μg l^{-1}), Cr (7.6 μg l^{-1}), Mn (760 μg l^{-1}), Fe (5400 μg l^{-1}), Ni (38 μg l^{-1}), Cu (160 μg l^{-1}), Zn (960 μg l^{-1}) and Cobalt, Cd and Pb were not detected in the AEA. In line with the former author, Plumlee et al. (2007) and Hageman et al. (2008) also reported low concentrations for these elements in leachates from ashes generated in several wildfires in California. In all the mentioned studies, the solubility of the studied elements were generally low when compared to their total concentrations in ash and the remobilization/recovery of chemical elements from ashes into the dissolved fraction of aqueous extracts varied among the analysed compounds (Plumlee et al., 2007; Hageman et al., 2008; Santín et al., 2015; Silva et al., 2015). The factors that probably influences the water solubility and their affinity towards the available organic matter (Koc) are the chemical specification of the element, the particle size and mineralogy of the ash, and in particular the pH of the solution, among others (Fedje, 2010). Based on this, it was expected that elements with higher solubility and lower Koc may exhibit higher concentrations in aqueous extracts.

Apart from metals, wildfires can also mobilised PAHs to aquatic systems. Besides the commonly described sources of PAHs to water bodies such as the atmospheric particulate matter deposition, the pollution of river and lakes by industrial effluents, the

municipal wastewaters discharges and oil spills (Manoli and Samara, 1999; Vila-Escalé et al., 2007), another important diffuse source of PAHs to the aquatic ecosystems are the wildfires (Gabos et al., 2001; Olivella et al., 2006; Vila-Escalé et al., 2007. Schäfer et al., 2010; Smith et al., 2011). As mentioned before, PAHs may be produced during combustion in forest fires and released into the atmosphere or deposited in ash and soil/sediments (Gabos et al., 2001; Kim et al., 2003; Vergnoux et al., 2011). These compounds can be transported into the groundwater by leaching through soil and into surface waters by surface runoff mainly adsorbed to ash and soil or by direct ash fallout in the water bodies, in a similar way as described for metals. In the surface water, PAHs can volatilize, photodegrade, oxidize, biodegrade by aquatic organism, bind to particulates or accumulate in organisms, depending on their physiochemical properties. In the sediments, PAHs can be biodegraded or accumulated in aquatic organisms (Yu, 2005). Because of their low solubility and high affinity for organic carbon, PAHs in aquatic systems are primarily found sorbed to particles that either have settled to the bottom or as suspended in the water column (Neff, 1979; Eisler, 1987, Yu, 2005). Eisler (1987) estimated that two-thirds of PAHs in aquatic systems are associated with particles and only one-third are present in the dissolved form.

Only few authors have studied the post-fire inputs of PAHs to water systems in burnt areas (Bundt et al., 2001; Rey-Salgueiro et al., 2004; Olivella et al., 2006; Vila-Escalé et al., 2007; Schäfer et al., 2010; Silva et al., 2014; Mansilha et al., 2015). Olivella et al. (2006) reported an increase in downstream PAH concentration ranging from 2 to 336 ng l^{-1} one month after a fire in Catalonia, Spain. These inputs in downstream were attributed to atmospheric deposition of ashes (in absence or low rain after the fire) and which was mainly due to 3-ring PAHs (PHE) and 4-ring PAHs (PYR). In the same study, after the first rain events the PAH concentrations were significantly reduced, ranging from 0.2 to 31 ng l^{-1}, due to the dilution effect, but PHE has continued to be the main PAH at nearly all sampling sites. In addition, the total PAHs concentrations measured in all samples remained within the European drinking water limits (Olivella et al., 2006). Vila-Escalé et al. (2007) observed an increase in post-fire inputs of PAHs to streams associated with increasing post-fire erosion in Catalonia, Spain. Fifteen months after the fire PAHs concentrations in water approached the background levels, however never reached levels of toxicological concern. Similar to the findings of Olivella et al.(2006), the 3-ring PAHs (PHE) was the dominant PAH in the dissolved phase samples, while in the particulate phase, the 4-rind PAHs (PYR) clearly dominated over the entire period studied by Vila-Escalé et al. (2007). Monitoring of PAHs concentrations in nine streams in Victoria, Australia, was undertaken by Schäfer et al. (2010), following a wildfire. The

estimated PAHs concentrations ranged from 0.1 to 9 ng l^{-1} in the streams, with the highest values found in the catchment extensively burnt. In line with the studies of Olivella et al. (2006) and Vila-Escalé et al. (2007), in general, compounds with 3-ring (PHE and FLU) and 4- rings (PYR) were the predominant (Schäfer et al., 2010).

A study conducted by Mansilha et al. (2015) to determine the influence of wildfire on PAHs levels in groundwaters in Serra da Estrela and Serra do Gerês, Portugal, found increases of one- to six-fold higher in groundwaters within the burnt area than in the control sites. In addition, burnt areas and control areas showed different profiles, with the levels of two to four rings compounds in the control area being markedly higher than the five to six rings. They also reported temporal reductions in PAHs levels, decreasing from 35 to 10 ng l^{-1}, which they attributed to the dilution effect (the campaign took place after intense precipitations conditions) rather than biodegradation, since these compounds are known to be persistent.

Hence, the findings of the available literature corroborates the existing concerns on the impacts of wildfires on aquatic systems and their role as a diffuse source of contaminants, mainly trace elements and PAHs.

Polycyclic aromatic hydrocarbons and metals have been found to produce a wide range of ecotoxicological effects in diverse aquatic organisms (Ali et al., 1999; Clemons et al., 1998; Yuan et al., 2003; Hellou et al., 2006; Zhou, 2008; Pereira et al., 2016). Likewise, contamination of water supply reservoirs by PAHs or metals constitute also a concerning risk to human health, either by direct consumption of water exceeding threshold values, or by the use of water for recreational activities as fishing and swimming (Zhou et al., 2008). Worth stressing in this respect is that some of the trace elements and PAHs are toxic at very low concentrations and have a strong tendency to persist, accumulate in the environment and biomagnify across the food chain (Boström, 2002; Rappaport et al., 2004; Cachot et al., 2006; Gupta and Singh, 2011).

Chemical analysis of the environment matrix such as soil, ash, sediment and water is the most direct approach to reveal the metal and PAHs pollution status in the environment. However, by itself chemical assessment is no longer considered meaningful or effective to infer about the possible deleterious effects and risks of such pollution to organisms and ecosystems (Fent, 2003; Zhou et al., 2008). In this way, the incorporation of ecotoxicological assays when addressing the risks posed by wildfires to the aquatic ecosystems provides a more integrative assessment since the immediate chemical analysis ignore bioavailability and the interactions between contaminants (Smolders et al., 2003).

Notwithstanding the likely risks posed by PAHs and metals to aquatic ecosystems (e.g. Kayal and Connel, 1995; Van der Oost et al., 2003; Tsidiris et al. (2006); Bihari et al., 2007; Alvarez et al., 2008; Hellou, 2011; Santos et al., 2016), there is a research gap concerning the mobilization of these compounds from burnt areas and their off-site effects on downstream aquatic ecosystems, with potential, deleterious consequences for the aquatic biodiversity.

Within the limited literature on this topic, Silva et al. (2015), found that aqueous extract of ash from a wildfire inhibited the luminescence of the bacteria *Vibrio fischeri* and induce decrease in the growth of the primary producers *Pseudokirchneriella subcapitata and Lemna minor*. Conversely, the ash extracts did not produce immobilization in *Daphnia magna* (zooplaktonic species), suggesting that short-term acute toxicity may be absent at higher trophic levels (Silva et al., 2015). Studies conducted by Pascoal et al. (2005), showed that PAHs compromise the ecological functions driven by aquatic microbial decomposers, particularly in the presence of other stressors. This is of particular concern due to the role that microbes play in aquatic food webs.

Thus, further studies on ecotoxicological assessment of the runoff from burned areas on aquatic biota are of high importance to understand the real implications of fires on aquatic resources, contributing therefore for an effective environmental risk assessment to ensure the resources sustainability. Moreover, in the light of the EU Water Framework Directive (WFD; European Commission, 2000) it is imperative to understand the implications of the input of metals and PAHs in the aquatic systems by wildfires, as the WFD requires the estimation and identification of significant diffuse sources of pollution.

Understanding the effects of wildfires on aquatic ecosystems is an evolving research field, with many questions to be resolved. Limitations of current knowledge, and the certainty that post-fire fire management must be based on scientific knowledge, highlight the need to better understand the on-and-off site effects of wildfires and their implications to the ecosystems sustainability.

1.4 Objectives and thesis structure

Understanding fire effects and patterns on terrestrial and water processes is critical for long-term forest planning and management. As a contribute for an effective environmental risk assessment to guaranty the resources sustainability, the main goal of this study was to clarify and evaluate the role of wildfires in the production and

mobilisation of metals and PAHs to terrestrial and aquatic systems. To this end, a stepwise approach was implemented, integrating the results from physicochemical characterization with laboratory toxicity testing using standard species from different trophic levels, allowing a solid combination of a variety of methods and techniques, which results in a holistic overview. To attain this general aim, specific objectives were defined:

I. Clarify the effects of wildfire on major and trace elements (metals) mobilisation, as well as the production and profiles of PAHs from soils and ashes of eucalypt and pine plantations;
II. Evaluate the influence of time-since-fire on trace metals and PAHs pathways;
III. To search for differences in major and trace elements and PAHs concentrations in soils and ashes between the two principals forest plantations in north western part of the Iberian Peninsula, i.e., eucalypt and maritime pine plantations
IV. Assess the toxicity of runoff from burnt areas on aquatic organisms from different trophic levels.

The data gathered throughout this study will be a valuable contribution for future implementation of restoration and management decisions environmental of burnt forest areas, especially regarding the contamination triggered by metals and PAHs.

To address the defined objectives this thesis is composed by six chapters. The first and sixth chapters concern the general introduction and final remarks of this thesis, respectively, while the other four are individual research papers per se, published or submitted to SCI journals. These papers have been organized to cope with the objectives of this work, thus providing the framework for the rest of this thesis.

Chapter II present a short-term study on the impacts of wildfire and rainfall on mercury redistribution from burnt soils and ashes. Mercury levels were quantified in soils and ashes from two different severity burnt areas (Ermida and S. Pedro do Sul, Portugal) and in contrasting slopes in terms of vegetation (eucalypt *versus* pine), as well as in an unburnt eucalypt slope. It presents also a heating experiment in order to clarify the effect of temperature on mercury release from soil.

Chapter III evaluate the role of wildfires as a potential non-point source of major and trace elements in the environment. To this end, levels of vanadium, manganese, cobalt, nickel, cooper, cadmium and lead were assessed in soils and ashes during fifteen

months after the fire (four sampling periods) in eucalypt- and pine-covered hillslopes, as well as in an unburnt eucalypt slope.

Chapter IV assess the inputs of PAHs after a wildfire in soils and ashes from burnt areas, since forest fires have been identified as an important source of PAHs. Therefore, the contents of fifteen PAHs, identified as priority by the United States Environmental Protection Agency, were analysed in soil and ash from two contrasting severity burnt areas, Ermida and S. Pedro do Sul, and contrasting slopes in terms of vegetation (eucalypt *versus* pine), as well as in an unburnt eucalypt slope. In order to evaluate the role of time-since-fire, this studied was performed during fifteen months after the fire.

Chapter V address the toxic effects of PAHs compounds present in the runoff from burnt areas through laboratory assays in which four aquatic species from distinct trophic levels (*Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Lemna minor* and *Daphnia magna*) were exposed to different dilutions of runoff. The runoff was collected in a recently burnt eucalypt area (Colmeal, north central Portugal) on two occasions, immediately after the fire and about one year later.

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Chapter II

Effects of wildfire on mercury mobilisation in eucalypt and pine forests

2. Effects of wildfire on mercury mobilisation in eucalypt and pine forests

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Abstract

Wildfires and subsequent rainfall play an important role in the redistribution of major, minor and trace chemical elements, which could be mobilised from burnt soils and ashes. In particular, the fire-induced release of mercury (Hg) into the environment is relevant to study due to its volatilisation and toxicity. However, the impact of wildfire followed by rainfall on mercury redistribution is poorly documented. Hence, eucalypt- and pine-covered hillslopes (1-3) were surveyed in two burnt areas (Ermida and S. Pedro do Sul, Portugal), as well as in a non-burnt eucalypt slope (Ermida). Top-soils (0-2 cm) and ashes were sampled 4 and 14 weeks after the fire, the latter following an episode of heavy precipitation. In order to clarify the effect of temperature on Hg release from the soil, a heating experiment was also conducted. Results revealed that 30% of the Hg retained in eucalypt soils was released by the fire, corresponding to a loss of 1.0-1.1 g Hg ha^{-1} of burnt soil. Levels in burnt eucalypt soils were twice the values registered in burnt pine soils for both fire severities. Mercury in ashes pointed to similar trend between the two types of vegetation. Results from the heating experiment with soil suggest that temperature reached in the wildfire was insufficient to destroy the most stable bonds of Hg-ligands. Mercury concentrations were not correlated with organic matter content, both in soils and ashes. It was estimated that rainfall caused a loss of 1.0 g Hg ha^{-1} from ashes and an uptake of 0.5 g Hg ha^{-1} of soil washed out. The difference between the previous estimations, 0.5 g Hg ha^{-1}, corresponds to the quantity of Hg transported to the surrounding area or eventually introduced into aquatic systems. Hence, the results obtained in the present study highlight the importance of wildfire and subsequent rainfall in the mobilisation of Hg in the environment.

Keywords: Wildfire, Mercury pathway, Runoff, Portugal

2.1 Introduction

Forest ecosystems undergo recurrent wildfires with serious impacts resulting from the destruction of vegetation coverage and severe changes in the properties of soil surface layers (e.g. Cerdà and Lasanta, 2005; Verma and Jayakumar, 2012; Rubenacker et al., 2012). It is well known that the magnitude of these changes depends mainly on the nature of the soil, type and biomass of the vegetation coverage, fire severity, ash production, and post-fire climate conditions such as timing and intensity of precipitation events (e.g. Ulery et al., 1993, Neary et al., 1999; Certini, 2005; Maia et al., 2012; Verma and Jayakumar, 2012). Wildfire can have profound effects on the functioning of the soil system (physical, chemical and biological elements), which can affect the sustained productivity and soil recovery of the burnt area (Certini, 2005; Doerr and Cerdà, 2005; Terefe et al., 2008; Verma and Jayakumar, 2012, Guénon et al., 2013). Both the direct effects of fire and also the overall changes to the ecosystem encountered in post-fire situation can lead to short-, medium- and long-term changes in the soil. One of the most intuitive and important soil change, during burning is the alteration of organic matter content (Certini, 2005). Organic matter (LOI) is a key factor for forest soil since it has a direct and/or indirect influences on the physical, chemical and biological characteristics of the soil. Fire affects soil organic matter both quantitatively and qualitatively and its changes may range from its almost total destruction to increased contents in surface layers due to external inputs, as partly charred litter and leaves, forest necromass and residual ash (González-Pérez et al., 2004; Knicker et al., 2005; Certini, 2005; Mataix-Solera et al., 2011). Among the effects of wildfires, it is important to recognize and underline its impact on the chemical availability of trace elements and pathways (Navrátil et al., 2009; Jakubus et al., 2010; Aref et al., 2011). In fact, wildfires can release and deposited metals on soil surface, either directly by combustion of vegetation and soil organic matter mineralization and leaching from ashes-soil interactions (Ulery et al., 1993; Antilén et al., 2006; Jakubus et al., 2010; Pereira and Úbeda 2010). Although ash produced by forest fires is a very heterogeneous material (depending strongly on plant species, type and part burnt, and combustion completeness), it primarily consists of oxides and hydroxides of base cations such as calcium, magnesium and potassium, but also includes trace metals (Ulery et al., 1993; Pereira et al., 2011a; Bodi et al., 2014). These compounds can be transported by overland flow and leached into the soil profile, and may have impacts on surface and groundwater chemical composition (Reneau et al., 2007; Cerdà and Doerr, 2008; Smith et al., 2011). Research studies on ash resulting from wildfires have been mainly focused

on soil erosion, physical and hydrological properties of the ash (e.g. Doerr and Cerdà, 2005; Quintana et al., 2007; Cerdà and Doerr, 2008; Balfour and Woods, 2013; Gabet and Bookter, 2011; Pereira et al., 2015). The chemical composition of wildfire ash has received comparatively little research attention (Ulery et al., 1993; Pereira et al., 2011b, 2012; Santín et al., 2012), especially in what concerns its trace metals constituents (Engle et al., 2006; Pereira and Úbeda, 2010; Pereira et al., 2011a).

The toxic element mercury (Hg) is of particular concern due to its volatility, toxicity, persistence and the relevance of the atmospheric compartment in the global Hg cycle (Cheng and Schroeder, 2000; Huang et al., 2011), showing potentially harmful impact on the environment and on human health (Alloway, 1995; Cheng and Schroeder, 2000, Huang et al., 2011). Water, soil and vegetation take up Hg species following wet and dry deposition, being the uptake by vegetation an important pathway by which metals enters the food chain (Friedli et al., 2001; Roy and McDonald, 2013). Once deposited, Hg may undergo methylation by biological processes into the highly toxic methyl mercury, a potent neurotoxin with a strong tendency to bioaccumulated and then biomagnified within the food chain (Boening et al., 2000; Caldwell et al., 2000; Gnamus et al., 2000; Amirbahman et al., 2004). Various studies have quantified anthropogenic emissions of Hg (e.g. Pacyna et al., 2003; Pacyna and Pacyna, 2002). However, natural emissions, particularly associated with wildfires that have been accounted as one of the important Hg non-point sources, are less well documented (Sigler et al., 2003; Biswas et al., 2008; Navrátil et al., 2009; Wiedinmeyer and Friedli, 2007). Forest soils accumulate Hg that is strongly sorbed to organic matter (Schwesig and Matzner, 2000; Biester et al., 2002; Grigal, 2003). The behaviour of Hg in soils is very complex and is mainly controlled by adsorption and desorption process, involving several chemical, physical and biological process (Reis et al., 2015). Its environmental and toxicological effects are however, highly dependent on Hg forms, as different species interact differently with soil constituents, exhibiting different behaviour, solubility, mobility and bioavailability (Biester et al., 2002; Bollen et al., 2008). Therefore, the identification of mercury species in soils contributes to understand the Hg behaviour and fate in the environment and its toxicity (Biester and Scholz, 1997; Rumayor et al., 2013). Methods based on species release from the matrix according to their desorption temperatures have been used to determine the speciation of Hg species in solid samples, including thermo-desorption (Biester et al., 2002; Rumayor et al., 2013, 2015; Reis et al., 2012, 2015).

During wildfires, Hg stored in vegetation, organic litter and soil tends to be associated with organic-rich ash and is released to the atmosphere predominantly in the form of gaseous elemental form (Hg^0), with up to 15% in the form of particulate mercury.

Mercury released from fires can have local, regional and global impacts, since Hg^0 can be readily transported to significant distances from the original source, whereas particulate Hg is likely to be deposited locally (Friedli et al., 2003a, 2003b; Sigler et al., 2003; Wiedinmeyer and Friedli, 2007). The amount of Hg released during a wildfire is limited by Hg accumulation in the ecosystem, particularly in the soil, prior to burning (Biswas et al., 2007, 2008). Large Hg losses result from the low volatilization temperatures (100-300°C) of Hg species. For example, during some wildfires, elevated soil temperatures are sufficient to decompose Hg-humic acid bond (200-330°C; Biester and Scholz, 1997). Experimental burns performed on soils showed a 79% decrease in Hg content after burning (Mailman and Bodaly, 2005). Recent studies have reported Hg release from soils burned during both prescribe fires (Woodruff et al., 2001; Harden et al., 2004; Engle et al., 2006) and wildfires (Biswas et al., 2007, 2008; Engle et al., 2006; Navrátil et al., 2009; Burke et al., 2010).

Fires, partitioning of Hg in soil and biomass, influence Hg pathways (Biswas et al., 2007, 2008; Witt et al., 2009). Additionally, runoff and wind can mobilise Hg bound to eroded soil, litter and ash particles, leading eventually to the transport of Hg to aquatic systems (Caldwell et al., 2000; Amirbahman et al., 2004). Engle et al. (2006) measured higher concentrations of Hg in surface ash one year following both wildfire and prescribed burns, and suggested that it was related to sorption of atmospheric Hg over the subsequent year, reflecting Hg's affinity for carbon in ash. However, very few studies have reported such effects on Hg pathways in burnt soils and ashes. Therefore, there is a need to better understand the terrestrial Hg cycle and, in particular, to investigate wildfires as a potential non-point source of Hg. Understanding this is important to predict the fate of atmospherically derived Hg in terrestrial ecosystems with important implications for the loading of Hg to water bodies (Obrist et al., 2009), which is reinforced by the knowledge about solute released due to the runoff and erosion, after wildfires (Lasanta and Cerdà, 2005).

Hence, this work aims to: (a) clarify the effect of a wildfire on mercury mobilisation from soils and ashes; (b) assess the influence of rainfall on the mercury pathway; and (c) evaluate the differences in Hg concentrations in soils and ashes between two vegetation types. For this purpose, soil and ashes were surveyed from eucalypt and pine forests burnt at different severities, as well as from a non-burnt eucalypt stand. Furthermore, in order to clarify the effect of temperature on Hg release a thermo-desorption assay of Hg from soils was performed in the laboratory.

2.2 Materials and methods

2.2.1 Characteristics of the study areas

Two recently burnt areas located in north-central Portugal: Ermida (E) (40° 43' 33'' N, 8° 20' 60'' W, 170 m a.s.l., Aveiro district); and S. Pedro do Sul (S) (40° 45' 35'' N, 8° 10' 04'' W, 370 m a.s.l., Viseu district) (Figure 5) were selected to conduct the present study. The wildfires destroyed 295 ha in Ermida (26th July 2010) and 5066 ha in S. Pedro do Sul (6th August 2010) (DUDF, 2011). The burnt areas were predominantly covered by eucalypt (*Eucalyptus globulus* Labill.) and maritime pine (*Pinus pinaster* Ait.) plantations. The mean annual temperature varies within similar ranges, 12-19 °C and 13-15 °C, at E and S, respectively. Both areas are characterised by a humid meso-thermal climate, with wet winters and prolonged dry and warm summers (Köppen Csb; DRA, 2002). Over the past 30 years, the annual rainfall at E and S ranged from 960 to 2531 mm and 523 and 1757 mm, respectively (SNIRH, 2013). The soils of the two study areas are mapped as humic Cambisols and, to a lesser extent, Leptosols, developed over granite, mainly for S. Pedro do Sul, and schist, mainly for Ermida (Cardoso et al., 1971).
Fire severity was assessed according to the methodology described in Shakesby and Doerr (2006), Keizer et al. (2008) and Keeley (2009). Hence, fire severity assessment was based on the degree of consumption of the canopies of 10 randomly selected trees as well as of the litter layer (partial or total), and on ash colour (black, grey and/or white), which was classified by visual inspection. The ash samples were ranked by shade (from dark to light) by comparing the samples with each other, without using the standard of greyness for comparison (Stronach and McNaughton, 1989; Parsons et al., 2010). The fire severity at each slope was determined at 5 points along a transect (Figure 5). In Ermida, the fire consumed the tree canopies only partially but the litter layer entirely, and produced black ashes; in S. Pedro do Sul, the fire totally consumed both the tree canopies and, the litter layer, and produced grey-white ashes. On the basis of these observations, fire severity was classified as moderate in the case of Ermida and as moderate to high severity in the case of S. Pedro do Sul.

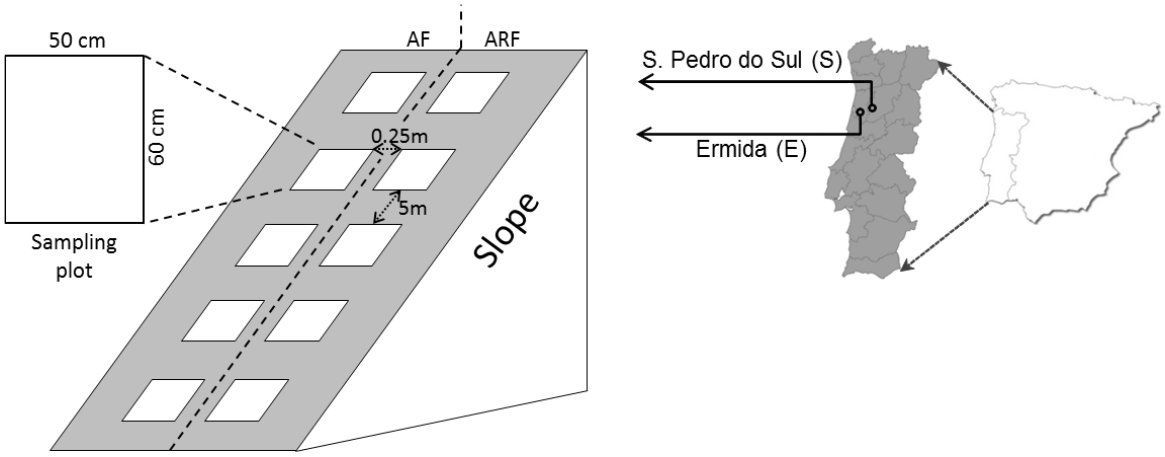

Figure 5 – Location of sampling sites (Ermida and S. Pedro do Sul) and design for soil and ash sampling.

2.2.2 Sampling design

Three hillslopes were selected in Ermida (E), both in eucalypt (BE) and pine (BP) burnt stands: E-BE1, E-BE2, E-BE3; and E-BP1, E-BP2, E-BP3 (Figure 5). The distance between slopes was around 50-m. A non-burnt eucalypt slope (E-NBE) was also selected in an adjacent area. Three burnt pine slopes were sampled in S. Pedro do Sul (S) (S-BP1; S-BP2 and S-BP3) distancing around 50-m, as well as one burnt eucalypt slope (S-BE1). On each slope, one transect was laid out across the full length of the slope section. Five equidistant points were established from the top to the bottom of each slope to take into account ash and soil spatial heterogeneity (Figure 5). At each of the five points of the sampling transect, a grid was laid out and a plot of 50x60 cm was sampled for soil and ash. At each sampling plot the entire ash layer was collected with a brush and a spoon. Ash samples were carefully collected in order to avoid mixture with soil. After the removal of litter, gravel and stones, the upper 2-cm of soil was sampled. Upper soil layers were selected based on previous studies that documented the preferential retention of Hg associated with soil organic matter (Biswas et al., 2007; Navrátil et al., 2009). Ash and soil samples were stored in plastic bag without air retention and taken to the laboratory for analysis. In the laboratory, those samples were air-dried, homogenised and sieved (2-mm mesh). Samples were stored in plastic bags at -18 °C until further analysis.

Sampling at Ermida was performed at two occasions- 4 weeks after the fire (AF) and 14 weeks after the fire (ARF) - whereas sampling at S. Pedro do Sul was limited to a

single occasion - 3 weeks after fire (AF). The AF samples in both areas did not reveal any signs of mobilization by overland flow. This agreed with the fact the first rainfall after the wildfires (as recorded by the network of rainfall gauges installed in the Ermida study area (Martins et al., 2013; Prats et al., 2014) between the first sampling in Ermida and in S. Pedro do Sul, and that it consisted of a small and low-intensity event (25 August 2010: P = 4.3 mm; I30 = 2.4 mm h^{-1}). By contrast, the second sampling in Ermida took place after a cumulative rainfall of 450 mm, with a maximum I30 of 30 mm h^{-1}. In total, 80 samples of ash and 90 samples of soils were analysed.

2.2.3 Heating experiment

A heating experiment was performed in the laboratory to follow the thermo-desorption or decomposition of Hg compounds (Biester et al., 2002; Lopez-Anton et al., 2010; Rumayor et al., 2013, 2015; Reis et al., 2012, 2015) in non-burnt and burnt eucalypt soils from Ermida. Twelve soil samples (approximately 10 g) of non-burnt and burnt eucalypt soils (Ermida) were placed in a controlled-temperature muffle furnace and subjected to the following increasing temperatures: 150 °C, 350 °C, 550 °C and 750 °C. These temperatures were chosen based on previous studies mentioned above. Soil samples were maintained at each temperature for two hours and thereafter three replicates were removed. Total Hg concentrations were determined in all samples exposed to this sequence of temperatures.

2.2.4 Analytical procedures

Soil pH and electrical conductivity (EC) were measured in Milli-Q water suspension of soil (1:5 v/v for pH and 1:5 m/v for EC) according to the method described in ISO 10390 (2005) and ISO 11265 (1994), respectively. For pH determination, the suspension was mechanically shaken for 60 min, allowed to settle for 2 h and, after shaking the soil suspension again, the pH was measured using a Metrohm 827 pH meter. For EC, the mixture was mechanically shaken for 30 min and filtered through a glass fiber filter (1.2 μm). Then, EC was measured in the filtrate with a Chemtrix type 7 conductivity meter. The pH and EC of ash suspensions were determined by mixing 6 g of ash with 36 ml of Milli-Q water, which was further mechanically shaken for 2 h and finally filtered through a 0.45 μm microfiber filter (Pereira et al., 2011b). The pH and EC of these suspensions

were measured as previously described. Organic matter content (LOI) was estimated by loss on ignition at 550 ºC for 4 h (Botelho da Costa, 2004).

In order to assess aluminium (Al) concentrations in soils, approximately 500 mg of dry soil samples (40 °C) were digested with HNO_3 (sp, 60% v/v) (95 °C for 2 h 45 min) in a DigiPrep HotBlock – SCP Science, redissolved with H_2O_2 (sp, 30% v/v) and Milli-Q water and heated for 2 h at 95 °C. After cooling, the solution was diluted to 50 ml with Milli-Q water (USEPA 3050B, 1996) and Al was determined by atomic absorption spectrometry (AAS- Perkin Elmer, Analyst 600). Aluminium in soils and sediments is commonly used as a proxy of particles nature and size since it is a key factor of trace elements accumulation (Windom et al., 1989).

Mercury analysis was performed using approximately 200 mg of dry soil and ashes (40 °C), which were thermally decomposed by controlled heating in an Hg analyser (AMA 254 LECO Instrument). The final decomposition products were passed through an Hg amalgamator heated to 700 °C and Hg^0 was released and detected by absorption spectrometry at 254 nm (Costley et al., 2000). Quality control of the analytical procedure was ensured by the analysis of international certified standards (MESS-3 and 277) and blanks that were prepared in a similar way to samples and run in parallel with them. Levels of Hg obtained in the reference materials were consistently within the ranges of certified values. Quantification limit was 22.7 ng g^{-1}.

2.2.5 Data analysis

Statistical analysis was performed with Sigmaplot 11.0. One-way analysis of variance was used to test whether non-burnt and burnt eucalypt soils were significantly different and a two-way analysis of variance was used to test statistical significance of the influence of the sampling periods (AF *versus* ARF), the vegetation type (eucalypt *versus* pine) and the sampling area (Ermida *versus* S. Pedro do Sul) on the pH, electrical conductivity, LOI, Al and Hg levels in soil and ashes. Specific differences between factors were detected *a posteriori* using the Tukey multicomparision test. Differences were considered significant at a $p<0.05$. Normality of data was checked by using the Shapiro-Wilk test or Kolmogorov-Smirnov, whether distribution was until 50 samples or was more than 50 samples, respectively. The homogeneity of variances of data was checked by using the Levene median test. When normality and homogeneity of variances were not achieved, data were transformed as Napierian logarithm (Ln) and square root. Enrichment factors for soil and ashes were calculated as the quotient

between Hg concentrations in samples collected following the rainfall episode (14 weeks after the fire) and 4 weeks after the fire at Ermida area. The Pearson or Spearman coefficients of correlation (whether variables were normally distributed or not, respectively) were used to determine any significant correlations between mercury concentrations in soil and ash and organic matter (LOI).

2.3 Results

2.3.1 Characteristics of soils and ashes

Table 1 presents the values of pH and electrical conductivity (EC) in soil and ash samples collected at Ermida and S. Pedro do Sul. On each slope of the selected areas, the variation coefficient of the means (n=5) of pH and EC were below 16 %. The soil collected in burnt eucalypt at Ermida showed values of pH and EC significantly (p<0.05) higher than the soil in non-burnt soil, with values 1.6 and 2.9 times greater, respectively.

As regard pH values between sampling surveys (AF *versus* ARF) in Ermida, the pH intervals in burnt soils AF (5.1-6.7) and ARF (4.6-6.5) and in ash 6.9-8.0 (AF) and 6.8-7.6 (ARF) were, in general, significantly (p<0.05) different either in soil or ash samples. Concerning the EC values, a similar pattern was observed in soil, 203-403 μScm^{-1} in AF and 125-368 μScm^{-1} in ARF, but pronouncedly significant differences were found in ash, 1050-1950 μScm^{-1} in AF and 125-360 $\mu S\ cm^{-1}$ in ARF. In general pH and EC in ash exceeded the values measured in soil.

Values of pH and EC of soil and ash from Ermida did not differ significantly (p>0.05) between the two vegetation coverages (eucalypt *versus* pine) (Table 1). A similar pattern was recorded for S. Pedro do Sul. Values of pH and EC of burnt soil and ash in Ermida were in general higher than in S. Pedro do Sul.

The Al content in non-burnt and burnt soils of the two areas, Ermida and S. Pedro do Sul, are depicted in Table 2. The Al values in Ermida varied between 2.0% and 2.9% for eucalypt soils and between 3.0% and 3.6% for pines soils. Furthermore, no significant (p>0.05) differences were found between: (1) non-burnt and burnt sites (NBE *versus* BE1, BE2, BE3); (2) vegetation type (eucalypt *versus* pine) and (3) sampling periods (AF *versus* ARF). A similar pattern was followed for Al in S. Pedro do Sul, with a tendency to have higher concentrations in pine soils (2.9-3.2%) than in eucalypt soils (2.0%), but without statistical differences (p<0.05).

Table 1 – pH and electrical conductivity (EC, μScm^{-1}) in soil and ashes of non-burnt (NBE) and burnt eucalypt (BE1, BE2, BE3) and maritime pine (BP1, BP2, BP3) slopes at Ermida (E) and S. Pedro Sul (S). Samples were collected 4 weeks after fire (AF) at E and S and 14 weeks later after a rainfall period (ARF) at E. Mean values ± standard errors are given. *Asterisks* indicate significant differences between non-burnt and burnt eucalypt samples. Different letters denote statistically significant differences ($p < 0.05$) between sites (for the same survey). Different symbols denote statistically significant differences ($p < 0.05$) between surveys (for each site).

Sampling Area	Type of sample	Site	Survey	pH	EC (μScm^{-1})
ERMIDA (E)	SOIL	NBE	AF	4.0 ± 0.01 ∇	120 ±5.0 ∇
			ARF	3.9 ± 0.09 ∇	125 ± 5.0 ∇
		BE1	AF	6.4 ± 0.10 ∗a∇	363 ±7.5 ∗ad∇
			ARF	6.0 ± 0.02 ∗a◊	220 ± 10.0 ∗a◊
		BE2	AF	6.0 ± 0.08 ∗b∇	290 ± 10.0 ∗b∇
			ARF	4.8 ± 0.04 ∗b◊	173 ± 2.5 ∗b◊
		BE3	AF	6.7 ± 0.07 ∗a∇	403 ± 2.5 ∗c∇
			ARF	6.5 ± 0.03 ∗c◊	368 ± 7.5 ∗c◊
		BP1	AF	5.8 ± 0.02 b∇	335 ± 15.0 a∇
			ARF	5.5 ± 0.04 d◊	125 ± 10.0 d◊
		BP2	AF	5.9 ± 0.06 b∇	393 ± 7.5 cd∇
			ARF	5.7 ± 0.04 d◊	310 ± 5.0 b◊
		BP3	AF	5.1 ± 0.07 d∇	203 ± 2.5 b∇
			ARF	4.6 ± 0.04 b◊	125 ± 5.0 d◊
	ASH	BE1	AF	7.2 ± 0.15 a∇	1950 ± 50 a∇
			ARF	6.8 ± 0.03 a◊	275 ± 25 a◊
		BE2	AF	6.9 ± 0.01 a∇	1050 ± 50 b∇
			ARF	6.8 ± 0.11 a∇	360 ± 10 a◊
		BE3	AF	8.0 ± 0.13 b∇	1550 ± 150 c∇
			ARF	7.6 ± 0.05 b◊	188 ± 3 a◊
		BP1	AF	7.3 ± 0.08 a∇	1375 ± 125 bc∇
			ARF	6.9 ± 0.11 ac◊	235 ± 5 a◊
		BP2	AF	7.9 ± 0.05 b∇	1600 ± 150 ac∇
			ARF	7.0 ± 0.10 ac◊	140 ± 10 a◊
			AF	7.1 ± 0.18 a∇	1350 ± 100 bc∇
			ARF	7.3 ± 0.13 bc∇	325 ± 35 a◊
S.PEDRO DO SUL (S)	SOIL	BE1	AF	5.6 ± 0.02 a	200 ± 0 a
		BP1	AF	5.0 ± 0.24 a	200 ± 5 a
		BP2	AF	5.0 ± 0.13 a	193 ± 3 a
		BP3	AF	5.4 ± 0.23 a	163 ± 8 a
	ASH	BE1	AF	6.8 ± 0.03 a	900 ± 100 a
		BP1	AF	6.7 ± 0.02 a	550 ± 50 a
		BP2	AF	6.7 ± 0.20 a	600 ± 50 a
		BP3	AF	6.4 ± 0.20 a	675 ± 25 a

Table 2 – Aluminium concentrations (Al, %) in soil of non-burnt (NBE) and burnt eucalypt (BE1, BE2, BE3) and maritime pine (BP1, BP2, BP3) slopes at Ermida (E) and S. Pedro Sul (S). Samples were collected 4 weeks after fire (AF) at E and S and 14 weeks later after a rainfall period (ARF) at E. Mean values ± standard errors are given. Different letters denote statistically significant differences ($p < 0.05$) between sites (for the same survey). Different symbols denote statistically significant differences ($p < 0.05$) between surveys (for each site).

Sampling Area	Type of sample	Site	Survey	Al (%)
ERMIDA (E)	SOIL	NBE	AF	2.0 ± 0.03 ∇
			ARF	2.1 ± 0.14 ∇
		BE1	AF	2.5 ± 0.11 a∇
			ARF	2.6 ± 0.22 a∇
		BE2	AF	2.5 ± 0.21 a∇
			ARF	2.9 ± 0.09 a∇
		BE3	AF	2.8 ± 0.04 a∇
			ARF	2.7 ± 0.25 a∇
		BP1	AF	3.2 ± 0.07 a∇
			ARF	3.0 ± 0.06 a∇
		BP2	AF	3.6 ± 0.11 a∇
			ARF	3.4 ± 0.18 a∇
		BP3	AF	3.1 ± 0.13 a∇
			ARF	3.2 ± 0.02 a∇
S.PEDRO DO SUL (S)	SOIL	BE1	AF	2.0 ± 0.09 a
		BP1	AF	2.9 ± 0.15 a
		BP2	AF	3.1 ± 0.06 a
		BP3	AF	3.2 ± 0.08 a

2.3.2 Mercury and loss on ignition in soil and ash after the fire

Figure 6 illustrate the values of Hg and loss on ignition (LOI) in the non-burnt soils from Ermida, as well as burnt soils and ashes from Ermida and S. Pedro do Sul collected 4 weeks after the fire (AF). The burnt eucalypt soils showed an increase of LOI in E-BE1 (34%) and E-BE2 (40%) in comparison to the non-burnt soils (E-NBE). Values of LOI in E-NBE and E-BE3 were not significantly different ($p>0.05$). An opposite pattern was observed for Hg concentrations in burnt eucalypt soils (mean values ranged between 61 and 62 ng g^{-1}), being significantly ($p<0.05$) lower than in non-burnt soils (88 ng g^{-1}). Additionally, no correlation (r=0.326, $p=0.157$) was observed between Hg levels and LOI. Mercury concentrations in burnt soils of eucalypt were significantly higher ($p<0.001$) than in soils from burnt pine slopes, both in Ermida and in S. Pedro do Sul. However, this pattern is not straightforward for the LOI values, as no significant differences ($p>0.05$) were found between E-BE1 and E-BP1 as well as between E-BE3 and E-BP2 in Ermida.

The absence of significant differences (p>0.05) between types of vegetation (eucalypt *versus* pine) for LOI values was also observed in S. Pedro do Sul. Furthermore, a week correlation was found for Hg concentrations and LOI levels (r=0.318, p=0.02).

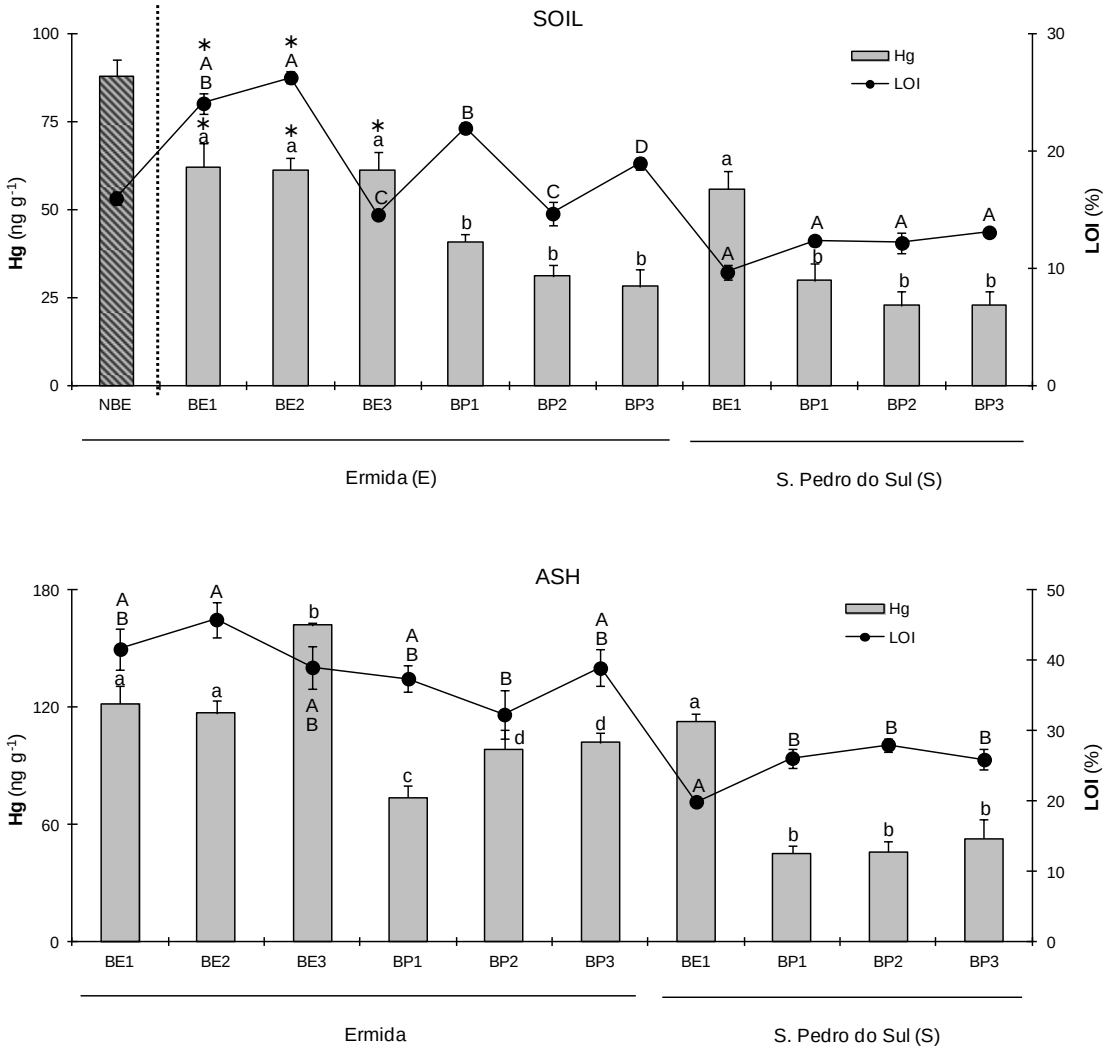

Figure 6 - Mercury concentrations (ng g^{-1}) and LOI (%) in soils and ashes collected at Ermida (E) and S. Pedro do Sul (S) 4 weeks after the fire (AF). Mean and associated standard errors are given. *Asterisks* indicate significant differences for Hg levels and LOI between non-burnt and burnt eucalypt areas at Ermida (BE1, BE2, BE3). Different lowercase and uppercase letters denote statistically significant differences (p < 0.05) between areas for Hg levels and LOI, respectively.

The comparison of Hg and LOI values in ashes from Ermida and S. Pedro do Sul is also illustrated in Figure 7. Mercury concentrations differed among the slopes, either between the distinct eucalypt slopes or between the pine slopes. For example, Hg values in the eucalypt slope E-BE3 were significantly (p<0.05) higher than in E-BE1 and E-BE2. Additionally, Hg levels in ashes from the pine slope E-BP1 were significantly

(p<0.05) lower than from E-BP2 and E-BP3. Likewise to Hg levels in soils, also ashes derived from eucalypt biomass were significantly (p<0.05) enriched in Hg in comparison to ashes from burnt pines at both areas (Ermida and S. Pedro do Sul). As for LOI values in ashes, we were unable to statistically distinguish between the different vegetation types (eucalypt *versus* pine) in Ermida, since significant differences (p< 0.05) were only found between E-BE2 and E-BP2. As for S. Pedro do Sul, LOI in ashes was significantly lower (p<0.01) in the eucalypt slope (S-BE1) than in the pine one's (S-BP1, S-BP2 and S-BP3). There were a poor correlation between Hg concentrations and LOI values (r=0.314, p=0.03). Ashes, as well as soils, from the moderate to high severity fire (HS) at S. Pedro do Sul showed lower levels of Hg compared to the moderate severity (MS) fire at Ermida (Figure 7). Despite this, significant differences (p<0.05) were only detected for samples collected in pine plots.

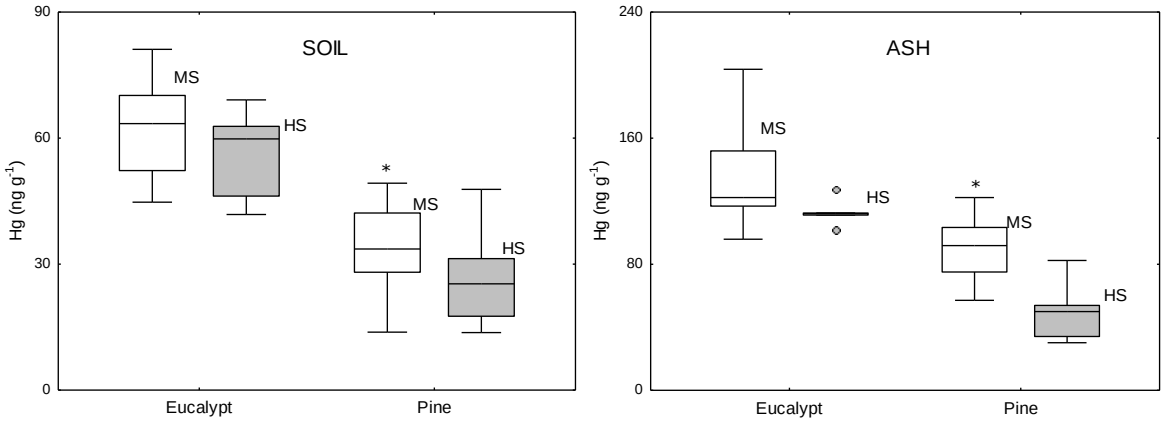

Figure 7 - Mercury concentrations in soils and ashes collected at Ermida (moderate severity – MS) and S. Pedro do Sul (moderate/high severity – HS). Samples were collected 4 weeks after the fire. Median, 25 and 75 percentiles, maximum, minimum and extreme values (o) are presented. *Asterisks* indicate significant differences between fire severities for both vegetation coverage.

2.3.3 Mercury and loss on ignition in soil and ash after the rainfall

Figure 8 shows Hg and LOI values in the non-burnt soils (E-NBE) and burnt soils and ashes of Ermida after the rainfall event (ARF). Burnt eucalypt soils contained significantly lower Hg concentrations (62-67 ng g^{-1}) than the corresponding non-burnt soils (86 ng g^{-1}). In opposition, burnt soils showed significantly higher LOI (23-26 %) than the non-burnt soils (16 %), with the exception of E-BE3. A lack of correlation (r=-0.206, p=0.378) was observed between Hg and LOI levels. The precipitation did not reduce the differences of Hg concentrations between eucalypt and pine areas that were observed

immediately after the fire (Figure 6; Figure 8). In fact, eucalypt soils exhibited significantly (p<0.05) higher levels of Hg than those in pine plantations. In general, these differences were also noticeable in ashes. Levels of LOI in soils differed among the slopes, either between the distinct eucalypt slopes or between the pine slopes. For example, LOI values in the eucalypt slopes (E-BE1, E-BE2 and E-BE3) were significantly (p<0.05) different between the three, while the pine slope E-BP2 were significantly (p<0.05) lower than from E-BP1 and E-BP3. Poor correlation was observed between Hg and LOI values in soils and ashes (r=0.434, p=0.02) and a moderate correlation in ashes (r=0.709, p=0.01).

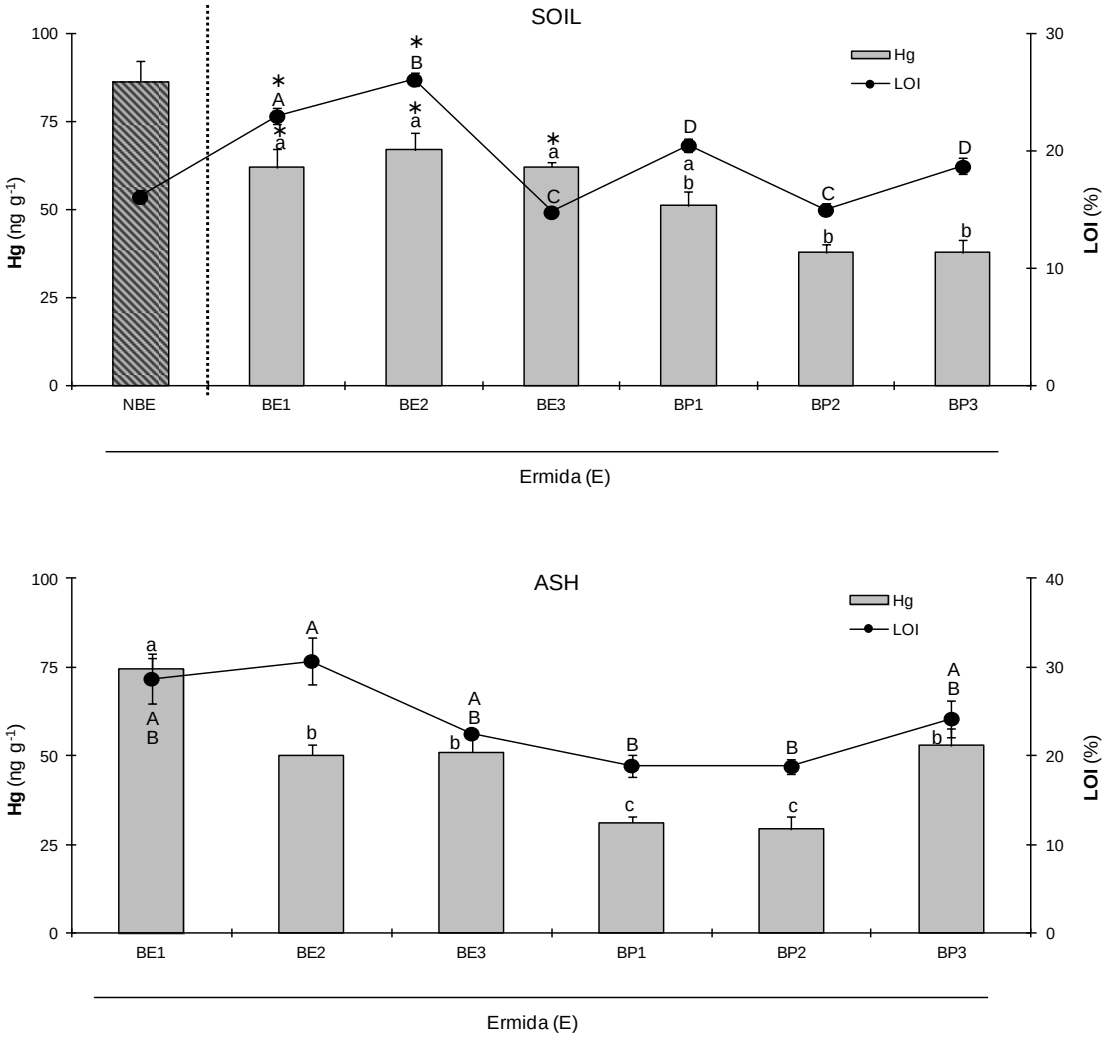

Figure 8 - Mercury concentrations (ng g^{-1}) and LOI (%) in soils and ashes collected at Ermida (E) 14 weeks after the fire and after a rainfall episode (ARF). Mean and associated standard errors are given. *Asterisks* indicate significant differences for Hg levels and LOI between non-burnt and burnt eucalypt areas in Ermida (BE1, BE2, BE3). Different lowercase and uppercase letters denote statistically significant differences (p < 0.05) between areas for Hg levels and LOI, respectively.

The effect of rainfall on Hg in burnt soils and ashes can be confirmed through the calculation of the enrichment factor (EF), as the ratio between Hg concentrations after and before the runoff period. Values of EF calculated for soils and ashes of eucalypt and pine stands from Ermida are presented in Figure 9. A contrasting situation was demonstrated by soils and ashes. Whereas EF_{soil} remained one or slight above (1.0-1.2), EF_{ash} were below one (0.3-0.6).

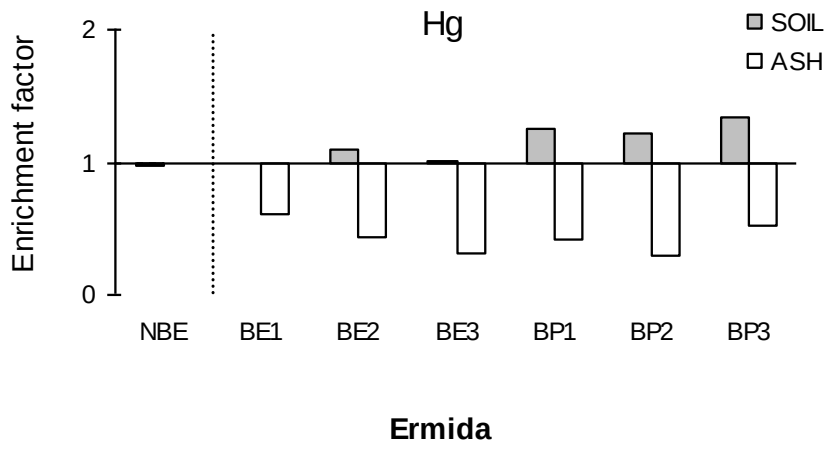

Figure 9 - Enrichment factors (EF) calculated by the quotient between Hg levels in the soils and ashes collected after rainfall (14 weeks after the fire) and 4 weeks after the fire at Ermida (eucalypt and pine areas).

2.3.4 Mercury release during the Ermida fire

Values of the amount of Hg stored in soils for the four slopes in Ermida, under post-fire and post-rainfall conditions are given in Table 3. Taking into account the mass of 2-cm upper soils collected in a plot with 3000 cm^2 and the respective Hg concentration, we find that the amount of Hg stored in the eucalypt burnt slopes in Ermida ranged from 1.8 to 1.9 g Hg ha^{-1}, which was lower than the observed at non-burnt eucalypt area, 2.9 g Hg ha^{-1} (Table 3). These quantities correspond to 34-38 % of the initial Hg in the soils. The average quantity of Hg release from the soil due to the wildfire (1.1 g Hg ha^{-1}) occurred at Ermida was calculated from the difference between the values in non-burnt soil (2.9 g Hg ha^{-1}) and burnt soils (1.8±0.05 g Hg ha^{-1}). It was also estimated the stored Hg in ashes AF and ARF, which ranged from 0.93-1.5 g Hg ha^{-1} and 0.11-0.17 g Hg ha^{-1}, respectively (Table 3). After the rainfall event it was observed a loss of 1.0 g Hg ha^{-1} from ashes and a slight enhancement of 0.5 g Hg ha^{-1} in the soils.

Table 3 – Mercury budget (g Hg ha^{-1}) in non-burnt (NBE) and burnt eucalypt soils and ashes (BE1, BE2, BE3) collected at Ermida 4 weeks after fire (Post-fire) and 14 weeks later after a rainfall period (Post-rainfall). Mean values ± standard deviation (SD) are given.

Sampling area	Site		Post-Fire		Post-Rainfall	
			Soil	Ash	Soil	Ash
Ermida (E)	NBE	(g Hg ha^{-1})	2.9	-	2.9	-
	BE1		1.9	0.93	2.3	0.17
	BE2		1.9	0.99	2.3	0.11
	BE3		1.8	1.5	2.2	0.14
		E-BE (Mean±SD)	1.8±0.05	1.1±0.31	2.3±0.08	0.14±0.03

2.3.5 Thermo-desorption of mercury from soil

Figure 10 illustrates the mercury concentrations in non-burnt and burnt eucalypt soils (collected 4 weeks after fire at Ermida) exposed to an increasing gradient of temperature. It was also plotted Hg levels in dry soil samples (40 °C) of Ermida that correspond to the initial reference values before the heating experiment. Mercury levels in all soil samples subjected to 40 and 150 °C were not significantly different. Moreover, burnt soils exhibited significantly lower ($p<0.05$) Hg levels at 40, 150 and 350 °C than non-burnt soils. The most pronounced loss of Hg was registered between 150 and 550 °C, through an apparent linear decrease with the temperature. However, previously burnt soils exhibited significantly lower Hg concentrations at 350 °C (18±5.3) than the non-burnt soils (34±3.3). Both non-burnt and burnt soils exposed to 550 °C showed residual values, corresponding to 3 and 4% of the initial Hg concentrations, respectively. After 750 °C, Hg levels in soils were below 1% of the initial Hg concentrations.

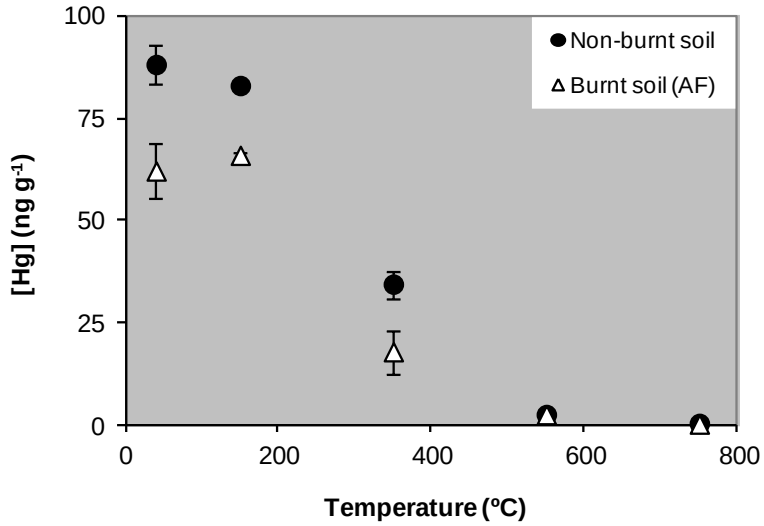

Figure 10 - Variation of Hg levels (ng g^{-1}) with exposure temperature (ºC) of non-burnt and burnt eucalypt soils from Ermida.

2.4 Discussion

2.4.1 Effects of fire and rainfall on physical and chemical properties of soils and ashes

The present study revealed an increment of EC and pH in burnt soils compared with non-burnt, which are in agreement with observations by several authors, who also reported that pH and EC values of burnt soils were greater than those of non-burnt soils (Hernández et al., 1997; Goforth et al., 2005; Quintana et al., 2007; Boerner et al., 2009; Granged et al., 2011a, b). Moreover, by comparing soil and ashes collected after the fire (AF) and later after significant rainfall (ARF) a decrease was observed for both pH and EC values. In fact, soil pH and EC is generally increased after forest fires at least during a few months later, which can goes up to 5 units after intense burning, in the case of soil pH and then return to similar levels to those of the corresponding unburnt soils (Cass et al., 1984; Ulery et al., 1993, 1995; Granged et al., 2011b). The higher values of pH and EC in soils and ashes collected after the wildfire can be explained by the release of ions from ash attributed to the mineralization of organic matter after fire, as well as by the mineralized nutrients contained in ash (Notario del Pino et al., 2008; Úbeda et al., 2009; Pereira et al., 2011b). Following the fire, substantial changes occurred in the soils of the burnt area. The vegetation, litter, and a portion of organic layer were at least oxidized by fire, which left behind the ash layer. As the ash was incorporated into the soil it originates

an increased in soil pH, as is supported by higher soil pH in burnt compared to the non-burnt soil (Amirbahman et al., 2004).

The soil pH and EC changes with time is mainly due to the combustion of organic matter, the amount and chemical composition of ash produced during fire, which released large quantities of inorganic ions (cations), oxide, hydroxide and carbonates in soils, a decrease in organic acids produced during the biological oxidation of organic matter in the detritus (Ulery et al., 1993, 1995; Kutiel and Inbar, 1993; Hernández et al., 1997; Certini, 2005; Boerner et al., 2009; Verma and Jayakumar, 2012). Decreases of pH and EC in burnt soils and ashes with the time are usually attributed to the leaching of ash during rainfall, which diminish the ions concentration in ash, namely soluble hydroxides and carbonates (Ulery et al., 1993; Certini, 2005). Temporal variations of pH and EC were much more accentuated in ashes than in burnt soil suggesting that alterations in the former are associated with ash properties. Reductions in soil pH and EC during post-fire were also detected by other authors (Kutiel and Inbar, 1993; Hernández et al., 1997; Kim et al., 2003; Granged et al., 2011b). Comparable ash pH values were observed in samples collected 2 and 3 days after the fire by Pereira et al., 2012 (pH = 6.89 - 6.98) and Pereira et al., 2014 (pH = 7.95) in a *Pinus pinaster* forest in Portugal, respectively. Similar values were also found by Kim et al. (2003) (pH = 7.24) and Goforth et al. 2005 (pH = 8.0) in samples collected 1 and 2 months after the fire, respectively. A temporal study with observations at 20 and 60 days post-fire revealed a decrease in ash pH from 12.0 to 9.3, respectively (Ulery et al., 1993). Electrical conductivity values found in ashes in the present study were also comparable to the findings of Pereira et al. (2011b; 2012) after a prescribed fire and those produced in laboratorial environments (Úbeda et al, 2009). In general, we found a tendency to have higher values in ashes than in soils, suggesting that ash deposition and heat associated with forest fire affect soil properties (Cass et al., 1984; Ulery et al., 1993; Kim et al., 2003; Goforth et al., 2005).

The low percentage of Al in the collected samples suggests that soils consisted mainly of a mixture of sand with a small proportion of fine particles.

2.4.2 Mercury retained in burnt soil

Total Hg in soils indicated that concentrations in burnt eucalypt soils were approximately 70% of the mercury contained in the corresponding non-burnt site. Current data are in line with other studies that have reported a decrease of Hg content in

soils after wildfires due to its release as Hg(0) (gaseous) or particulate mercury (Engle et al., 2006; Biswas et al., 2007, 2008; Navrátil et al., 2009; Obrist et al., 2009; Burke et al., 2010). This pattern was consistent with other investigations conducted in soil samples that included litter, humus and organic soil (Amirbahman et al., 2004; Mailman and Bodaly, 2005). Comparisons between Hg concentration in non-burnt and burnt soils provides insight on the effect of wildfire on Hg distribution and cycling in forest (Engle et al., 2006). The higher Hg values found in non-burnt than burnt soils in the present study demonstrate that some of the Hg that was accumulated prior to burning was lost from soil to the surrounding environment when burnt, confirming the potential of wildfire to directly volatilize Hg, as a result of their low volatilization temperatures. Since, most of the Hg species are volatilized between 100-300 °C (Biester and Scholz, 1997) and during the wildfire in Ermida, the elevated soil temperatures sufficient (higher than 150°C) to mobilize the Hg compounds, which included conversion to gaseous forms and subsequent emission (Hg released from fire is predominantly as Hg^0, with up to 15% in the form of particulate mercury; Friedli et al., 2001, 2003a, 2003b), vaporization of volatile compounds, and release as / on ash particles (our results showed higher levels of Hg in ashes than in soils). Another possibility is the conversion to more soluble forms that can be leached into the soil (Gimeno-García et al., 2000; Engle et al., 2006).

The loss of 1.0-1.1 g Hg ha^{-1}due to the wildfire occurrence indicates that only a small portion of the Hg present in the upper 2- cm layer of soil was removed, where most of the Hg is likely to be present. Due to volatilisation of Hg(0), most of the mercury forms present in top soils during wildfire tend to be sequentially released as temperature increase (Biester and Scholz, 1997; Reis, 2013). In fact, the progressive release of Hg from the soil was confirmed in the present study by the heating experiment. Above 150 °C the bonds of mercury-ligands are progressively destroyed up to 550 °C. The high proportion of Hg retained in burnt soils (70%) after the wildfire, suggests that temperature from the 2-cm upper layer of soils did not reach those elevated values (it should have been above 150 °C and below 350 °C). Furthermore, the Hg levels in non-burnt soils and burnt soils after being submitted to temperatures of 150 and 350°C were 83 and 34 ng g^{-1} and 24 and 18°C, respectively, meaning that the temperature reached by the fire must have been above 150 °C and below 350 °C. Mercury concentration in the non-burnt and burnt soil (88 and 62 ng g^{-1}, respectively) was approximately the double and triple of the values found for the those samples, after being submitted to the temperature of 350 °C, respectively. According to this variation of Hg levels in non-burnt and burnt soils with temperature, one may speculate that the average temperature of the

upper soil layer during fire reached values high enough to destroy the labile forms of Hg, which are released above 100 °C and below 200 °C, like Hg^0 and $HgCl_2$, while most of Hg-humic acid bonds and HgS remained unbroken (Biester and Scholz, 1997). This hypothesis is probably valid to the other fire scenario (moderate to high fire severity occurred at S. Pedro do Sul), since Hg values in eucalypt and pine soils were comparable to levels recorded in the respective soils from Ermida.

2.4.3 Association of mercury with organic matter

In general, a similar pattern to pH and EC was followed by LOI (with the exception of ERM-BE3), where a steep increase approximately 35% was recorded for burnt eucalypt after the fire, in comparison with the non-burnt, that was maintained throughout the post-fire observation period. Our results confirms reports elsewhere in the literature, that suggested new inputs as partly charred litter and leaves, forest necromass and residual ash (Gimeno-García et al., 2000; Kim et al., 2003; Pardini et al., 2004; Knicker et al., 2005; Jovanovic et al., 2011). However, it is not unusual to find a decrease in LOI after fire due to the volatilization or oxidation of the organic matter, depending on the soil temperature reached during the fire (Terefe et al., 2008; Aref et al., 2011; Granged et al., 2011a). The extremely high values of LOI in ashes collect IAF, can be attributed to the incomplete vegetation combustion, as suggested by the ash dark color (Goforth et al., 2005).

Soil organic matter (SOM) has been considered a major factor to explain the retention of Hg in soil, mainly due to its high affinity with functional groups rich in sulphur and carboxyl present in soils (Engle et al., 2006; Biswas et al., 2007, 2008; Navrátil et al., 2009; Obrist et al., 2009). However, Obrist et al. (2012) also reported considerably higher soil carbon pools that were not associated with corresponding higher Hg stocks. The thermo-desorption assay performed in this study using non-burnt soils suggests that most of Hg was associated with ligands that were not destroyed during the wildfire (Biswas et al., 2007; Reis, 2013). Additionally, the absence of correlation ($r=0.326$, $p=0.157$) between Hg and LOI in burnt soils meaning that the organic matter content is insufficient to explain the Hg partitioning. It is likely the consumption of organic matter by the fire happens in parallel with input of LOI from the incomplete combustion of belowground biomass and litter. Consequently, Hg tends to be redistributed in the soil or released to the atmosphere. That tendency for a non-equilibrium situation is illustrated by burnt eucalypt soils in E-BE1 and E-BE2 that have 50% more LOI than the non-burnt

eucalypt soils. Additionally, due to the ash content accumulated in the top of the burnt soils, the Hg may bind with ash and with the remaining charcoal, therefore explaining the low concentrations of Hg observed in the burnt site.

The mercury concentration in burnt soils of areas colonised by eucalypt were approximately twice the values measured in burnt pine soils. That difference occurs for both surveyed areas, Ermida and S. Pedro do Sul, despite the different fire severities in the two study areas (Figure 7). Those differences were not related to the enhancement of LOI (r=0.318, p=0.02). Furthermore, they are unlikely related to the grain size distribution of the soil since aluminium, which is a proxy for particle size in soil (Windom et al., 1989), is not significantly different (p>0.05) in the two study areas. Additionally, the influence of different plant species on the soil characteristics is well documented (Mailman and Bodaly, 2005; Obrist et al., 2012). Nevertheless, further studies should confirm whether eucalypts, in comparison to pines, create conditions that favour Hg retention in soil.

Mercury concentrations in ashes were twice the values measured in soils. Furthermore, Hg in ashes collected in the eucalypt plots were significantly (p<0.001) higher than in the pine stands, both in Ermida and S. Pedro do Sul. These differences are in line with the values measured in soils. The quantity of organic matter in ashes did not explain those differences, since Hg is poorly correlated with LOI in ashes from the two burnt areas (r=0.314, p=0.03). Assuming that most of the collected ashes derived from the combustion of aboveground plant parts of eucalypt and pine, it seems that eucalypts accumulate more Hg than pines at Ermida. This difference is obvious from the box-plots of Hg levels for soils and ashes from the two plants coverage under both fire severities (Figure 7).

2.4.5 The effect of rainfall after the fire

Several studies have clearly pointed out that heavy rainfall and the subsequent surface runoff play an important role in soil erosion and therefore on transport of particles to downstream aquatic systems (Caldwell et al., 2000; Cannon et al., 2001; Moody et al., 2013; Malvar et al., 2011; Prats et al., 2014). These events are considered important inputs of contaminants from diffuse sources to rivers and estuaries (Schroeder and Munthe, 1998; Kelly et al., 2006; Obrist et al., 2009; Burke et al., 2010). Particularly, the first heavy rains after a wildfire caused an accentuated surface washing over the burnt areas of Ermida, which was attenuated over time (Martins et al., 2013; Prats et al.,

2014). In general, this leads to a significant decrease of pH and EC in soils and ashes. The effect of rainfall showed a contrasting situation between soils and ashes, since EF_{soil} remained one or slight above and EF_{ash} were below one. That Hg reduction in the ashes, which reached 30 to 60%, was accomplished with a decrease of LOI between 50 to 70%. Presumably, runoff promoted the removal of particulate Hg or solubilised the forms attached to the organic matter in ashes. That wash over showed to be effective in soils, since EF were near one. Values slightly above one may indicate the transference of Hg from ashes to soils. On the other hand, the atmospheric deposition of Hg on soil, with the rain (Lawson and Mason, 2001) is less plausible since ashes demonstrate a strong decrease in Hg concentrations.

Mercury concentrations in ash samples collected after the wildfire (AF) were much higher (< 74 ng Hg g^{-1}; Figure 6) compared to the one´s reported by Friedli et al. (2001) (< 7 ng Hg g^{-1}). These measurements of Hg concentrations were conducted on ash from controlled laboratory burnt experiments using vegetation and litter, which differs from ash produced following wildfire, due to the different combustion conditions, material and temperature reached during the experiment (Pereira et al., 2011a). High Hg levels still found in ash collected ARF (from 30 to 75 ng Hg g^{-1}; Figure 6) which are comparable but somewhat higher than the values found by Engle at al. (2006) in ashes collected 1 year after a forest prescribe fire and a wildfire (24.7 and 39.2 ng Hg g^{-1}, respectively).As mentioned before, Hg levels in ashes samples collected ARF were lower than in ashes collected AF. This temporal decrease of Hg in ashes can be attributed to their wash out by runoff and leaching into soils below after the first rainfalls. This hypothesis is reinforced by data of soil Hg concentrations where a slight increased ARF was observed.

2.4.6 Estimation of mercury budget

Burnt eucalypt slopes in Ermida were characterized by 1.1 g Hg ha^{-1} less than the control (non-burnt eucalypt slope) and this difference can be interpreted as Hg loss from the soil by fire. That quantity is in the same order of magnitude as values reported in literature for: a conifer wildfire (2.2-4.9 g Hg ha^{-1}), a prescribed conifer fire (2.2-5.1 g Hg ha^{-1}) (Engle et al., 2006), and a Quebec boreal forest (1.5 g Hg ha^{-1}) (Sigler et al., 2003). Estimated values were higher than those reported for a desert sagebrush fire (0.36 g Hg ha^{-1}) (Engle et al., 2006). In addition to 1.1 g of Hg released per ha to the atmosphere, Hg was also washed over following heavy rainfall. Rainfall caused a loss of 1.0 g Hg ha^{-1} from ashes and the slight enhancement of 0.5 g Hg ha^{-1} in the soils, meaning that it may

have been a possible transference of Hg from ashes to soils. Therefore, the net value of 0.5 g Hg ha^{-1} could be lost to the adjacent terrestrial areas or eventually introduced into freshwater systems.

2.5 Conclusion

Results of this study provided the main findings:

- 30% of the Hg retained in eucalypt soils escaped during a wildfire, corresponding to a loss of 1.0-1.1 g Hg per hectare. Temperature reached in the wildfire was insufficient to destroy the most stable bonds of Hg-ligands, as pointed by the thermo-desorption experiment.
- Soils and ashes from eucalypt hillslopes were consistently enriched in Hg relatively to pine areas. Ashes exhibited higher organic matter and Hg relatively to soils.
- The rainfall after the fire has a key role in the washout of Hg from ashes deposited on the soil surface.
- This study points out the importance the importance of wildfire and subsequent rainfall in the mobilisation of mercury in the environment.

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Chapter III

Major and trace elements in soils and ashes of eucalypt and pine forest plantations in Portugal following a wildfire

3. Major and trace elements in soils and ashes of eucalypt and pine forest plantations in Portugal following a wildfire

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Abstract

Wildfires can play an important role in the environmental distribution of major and trace elements, including through their mobilization by fire-induced runoff and associated transport of soil and ash particles. In particular, fire-induced inputs of these elements into the environment are relevant due to their toxicity and environmental persistence. This study aimed to evaluate the role of wildfire and time-since-fire on the redistribution of major and trace elements, which is a topic poorly documented. To this end, levels of V, Mn, Co, Ni, Cu, Cd and Pb were assessed in soil and ash samples collected immediately following a wildfire in north-central Portugal as well as 4 (after the first post-fire rainfall events), 8 and 15 months later. The role of forest type was determined by sampling burnt eucalypt and pine plantations. The main findings of this study were the following: (1) levels of V, Mn, Ni, Cd and Pb were consistently higher in the burnt than unburnt soils, while levels of Co and Cu revealed no differences; (2) time-since-fire affected major and trace elements in three different ways: concentrations of Mn and Cd declined abruptly after the first rainfall events whilst levels of V, Co and Ni increased during the first 8 months and levels of Cu and Pb hardly changed during the study period; (3) all studied elements revealed peak concentrations in the ashes immediately after the fire, which then declined sharply four months later; (4) levels of Co and Ni soils and ashes were higher at the pine plantations than at the eucalypt plantations. This study highlighted the role of wildfire in enhancing levels of major and trace elements in ashes and topsoil of forest plantations and their mobilization within the first year after fire, pointing towards recently burnt forest areas as a potential source of environmental contamination.

Keywords: Forest fire, Topsoil, Time-since-fire, Mobilization

3.1 Introduction

Forest fires have been a frequent phenomenon in the Mediterranean countries of southern-Europe over the past decades, devastating in Portugal, for example, an average of 144 000 ha per year and over 400 000 ha in a dramatic year such as 2003 (ICNF, 2015). In the future forest fires are likely to become increasingly frequent and severe, not just because of the foreseen increase in fire-propitious meteorological conditions due to climate change (IPCC, 2013) but also because the underlying causes are in part societal, reflecting rural depopulation, land abandonment and afforestation with fire-prone species (Moreira et al., 2009, 2011; Carmo et al., 2011).

Wildfires constitute an environmental problem that raise widespread concerns as they have adverse and diversified impacts on atmospheric, terrestrial, and aquatic compartments, with economic and social repercussions (e.g., Smith et al., 2011; Verma and Jayakumar, 2012; Liu et al., 2014).

It is well known that wildfires can strongly modify the abiotic and biotic characteristics of soil, altering its physical, chemical and biological properties (e.g., Certini, 2005; Ekinci et al., 2006; Shakesby, 2011; Ginzburg and Steinberger, 2012). Fire effects on deposition, mobilization and fate of contaminants, in particular trace metals, however, have been a relatively neglected issue. Wildfires have been found to release and deposit metals on the soil surface, either directly by combustion of vegetation and mineralization of soil organic matter or indirectly through interactions of ashes with the underlying-soil (Parra et al., 1996; Jakubus et al., 2010; Bogacz et al., 2011; Jovanovic et al., 2011; Costa et al., 2014). Both processes can lead to the addition of potentially significant amounts of these elements in the soil and, thereby, can alter its chemical properties (Ulery et al., 1993; Antilén et al., 2006; Jakubus et al., 2010; Pereira and Úbeda 2010).

The surface of the soil is the most affected by fire and, in particular, by ash deposition (Mandal and Sengupta, 2006). Ash produced by forest fires is typically a very heterogeneous material, composed mainly of oxides and hydroxides of base cations (Ca^{2+}, Mg^{2+} and K^{+}) and, to a lesser extent, by major and trace metals that can result in soil contamination (Someshwar 1996; Pitman 2006; Plumlee et al, 2007; Campos et al., 2015). Furthermore, these contaminants can be leached into the soil profile or transported downslope and downstream by runoff, impacting surface water and groundwater bodies (Reneau et al., 2007; Cerdà and Doerr, 2008; Smith et al., 2011; Campos et al., 2012). These post-fire pollutant fluxes will vary depending on a variety of factors such as fire intensity, vegetation composition and plant parts burnt (i.e. leaves,

bark, twigs, litter), soil type, meteorological conditions before after and during fire, and post-fire hydrological and erosion response (Ulery et al, 1993; Someshwar 1996; Werkelin et al., 2005; Wang and Dibdiakova, 2014; Machado et al., 2015).

Trace metals are of great environmental concern due to their high toxicity, environmental persistence and tendency to bioaccumulate in the food chain (Adriano, 2001; Ignatavièius et al., 2006). While a number of metals such as Cu, Zn and Fe are essential to living organisms, for example for protein structure stability, electron transfer reactions and gene regulation (Siegel, 1973), others such as Cd, Pb and Hg may displace or replace essential metals and interfere with the proper functioning of enzymes and associated cofactors, causing toxic effects in organisms (Siegel, 1973; Gifford et al., 2004). Even the former metals, however, can cause toxic effects if present in elevated concentrations (Alloway, 1995; Sunda and Huntsman, 1998; Gifford et al., 2004). Diffuse inputs of trace metals into the environment through, for example, forest fires are also undesirable because of their tendency for accumulation over time, with long lasting effects after elimination of their major sources (Chen et al., 1997; Sun et al., 2001; Bretzel and Calderisi, 2006; Hjortenkrans et al., 2006). Mobilization of major and trace metals by lateral transport, either in dissolved or particulate form, is likely to be significant in recently burnt areas, as fire typically enhances overland flow generation and the associated transport of ash and soil particles to downstream water bodies during periods that can last several years (Cannon et al., 2001; Lasanta and Cerdà, 2005; Malvar et al., 2011; Moody et al., 2013; Prats et al., 2014). Furthermore, leaching of major and trace elements into the soil can constitute risks of groundwater contamination and aquatic systems (Burke et al., 2010; Smith et al., 2011; Costa et al., 2014). This is of special relevance in Portugal, as forest areas are typically regarded as groundwater recharge zones.

Few studies have reported on the presence of major and trace elements in recently burnt forest soils and their evolution with time-since-fire. Therefore, there is a need to investigate wildfires as a potential non-point source of major and trace elements in the environment. To address this research gap, the present work aimed to: (a) clarify the effects of wildfire on the levels of major (Mn) and trace (V, Co, Ni, Cu, Cd and Pb) elements in the topsoil and ashes of eucalypt plantations; (b) evaluate the influence of time-since-fire on major and trace elements pathways; and (c) compare the major and trace elements contents between the two principal forest plantations in the north-western part of the Iberian Peninsula, i.e. eucalypt and maritime pine plantations.

3.2 Materials and methods

3.2.1 Characteristics of the studied area

This study was carried out in a recently burnt area located in north-central Portugal, near the parish of Ermida, Aveiro district (40° 43' 33'' N, 8° 20' 60''W, 170 m a.s.l.; Figure 11). The selected area was burnt by a wildfire on 26 July 2010, which destroyed an area of 295 ha (DUDF, 2012), predominantly covered by eucalypt (*Eucalyptus globulus* Labill.) and maritime pine (*Pinus pinast*er Ait.) forest plantations. The study area is characterised by a humid meso-thermal climate, with wet winter and prolonged dry and warm summer (Köppen Csb; DRA, 2002). Mean annual temperature at the nearest climate station (Castelo Burgães: 40º 51' 10''N, 8º 22' 44''W at 306 m a.s.l.) was 14.9 ºC (SNIRH, 2011: 1991-2011), while annual rainfall at the nearest rainfall station (Ribeiradio: 40º 73' 65''N, 8º 30' 08''W at 228 m a.s.l.) was, on average, 1609 mm but varied markedly between 960 and 2530 mm (SNIRH, 2011: 1991-2011). The soils of the study area were mapped as humic Cambisols and, to a lesser extent, Leptosols, developed over granite and schist (Cardoso et al. 1971). The overall fire severity in the study area was classified as moderate, according to the methodology described in Shakesby and Doerr (2006), Keizer et al., (2008) and Keeley (2009).

3.2.2 Sampling design

A total of six hillslopes were selected in the burnt area of Ermida, three in eucalypt (BE1, BE2 and BE3) and three in pine (BP1, BP2 and BP3) stands. The distance between slopes was around 50-m and their inclination ranged from 22 to 28 º. An unburnt eucalypt slope (UBE) was selected in the immediate surroundings of the burnt area, for assessing the wildfire effects. On each slope, a transect was laid out over the full length of the slope section at the first sampling occasion and then shifted across the slope at subsequent sampling occasions. Five equidistant points were sampled along each transect to account for spatial variability in ash and soil characteristics (Figure 11). At each sampling point, a plot of 50x60 cm was laid out to collect first the entire ash layer within this plot area, using a small brush and spoon and with special care to avoid the admixture of the upper soil layer, and then the upper 2-cm of the mineral soil, after eliminating gravel and stones.

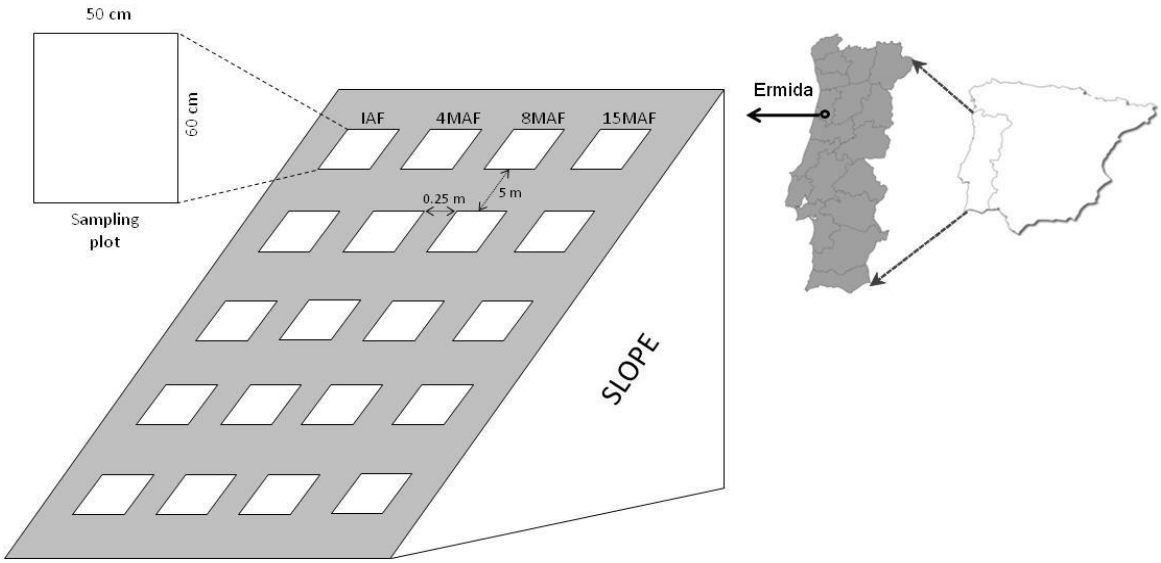

Figure 11 – Location of sampling area (Ermida) and design for soil and ash sampling (IAF- immediately after the fire; 4, 8 and 15MAF- 4, 8 and 15 months after the fire, respectively).

Sampling was carried at four occasions after the wildfire, namely: immediately and 4, 8 and 15 months after the fire (hereafter designated as IAF, 4MAF, 8MAF and 15MAF, respectively). Total rainfall between the fire and the first sampling occasion was negligible (4.3 mm), while cumulative rainfall between subsequent sampling occasions was 461, 739 mm and 272 mm, respectively (Figure 12). The first hydrological year after the wildfire was about an average year in terms of rainfall (-10 %), while the second year was relatively dry (-30 %) (Keizer et al., 2015).

In the laboratory, soil and ash samples were air-dried, homogenised, sieved (2 mm mesh), and then stored in plastic bags at -18 °C until further analysis.

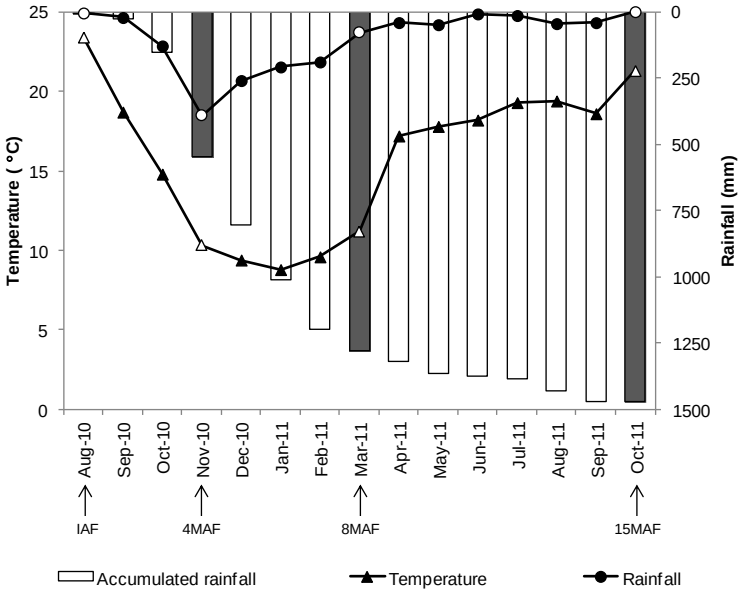

Figure 12 – Mean monthly temperature, monthly rainfall amounts and the accumulated rainfall at the study area (Ermida) during the first 15 months after the wildfire in July 2010. The grey column and the white circle depicts the month of the sampling soils and ashes (IAF- immediately after the fire; 4, 8 and 15MAF- 4, 8 and 15 months after the fire, respectively).

3.2.3 Analytical procedures

Soil pH and electrical conductivity (EC) were measured in Milli-Q water suspension of soil (1:5 v/v for pH and 1:5 m/v for EC), according to the methods described in ISO 10390 (2005) and ISO 11265 (1994), respectively. Organic matter content (OM) was estimated by loss on ignition at 550 ºC for 4 h (ISO 11465, 1993; Botelho da Costa, 2004).

Approximately 500 mg of dry ash and soil samples (40 °C) were digested with HNO_3 (sp, 60% v/v) (95 °C for 2 h 45 min) in a DigiPrep HotBlock – SCP Science, redissolved with H_2O_2 (sp, 30% v/v) and Milli-Q water, and heated for 2 h at 95 °C. After cooling, the solution was diluted to 50 ml with Milli-Q water (USEPA 3050B, 1996). Concentrations of vanadium (V), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), cadmium (Cd) and lead (Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS - Thermo Elemental, X-Series), while aluminium (Al) concentrations were determined by atomic absorption spectrometry (AAS- Perkin Elmer, Analyst 600). Quality control of the analytical procedure was ensured by the analysis of certified reference materials (CRM: BCR- 142R, BCR-143R and BCR-176R) and by testing every 10^{th} sample in duplicate. Blanks were prepared following the same

analytical procedures and run in parallel with the CRM and samples. Levels of the analysed elements in the CRM were consistently within the ranges of the certified values.

3.2.4 Data analysis

One-way analysis of variance was used to test whether pH, EC, OM content and major and trace element concentrations in unburnt and burnt eucalypt ashes and soils were significantly different, while two-way analysis of variance was used to test for significant differences among the four sampling occasions (IAF, 4MAF, 8MAF and 15MAF) as well as between the two forest types (eucalypt vs. pine). Specific differences between these factors were analysed using the Tukey multiple comparison test. Normality and homogeneity of variances of the data were assessed using the Shapiro-Wilk test and the Levene median test, respectively. When normality and/or homogeneity of variances failed by these tests, data were transformed as Napierian logarithm (Ln). The Spearman rank correlation coefficient was used to relate the concentration of major and trace elements to the general soil and ash properties of pH, EC and OM. All statistical tests were carried out using a α of 0.05.

The effect of wildfire on the availability of major and trace elements in soils was estimated by means of fire-related enrichment factors (EF_{WF}), computed as the ratio between the major and trace elements concentrations (C) in burnt eucalypt soils (BE) and their concentration in unburnt eucalypt soils (UBE). This was done for each sampling occasion separately. The enrichment factor expresses the relative abundance of a chemical element in a matrix compared to a reference matrix. In this study we used the unburnt eucalypt soil as the reference value (considered representative for the uncontaminated soil). Also the enrichment factors due to rainfall (EF_R) were estimated, using the ratio of the concentrations 4 months after the fire relative to those immediately after the fire. This was done for ashes as well as for soils.

3.3 Results

3.3.1 Physicochemical characteristics of soils

The average values and standard deviations of the pH, electric conductivity (EC) and organic matter (OM) content of the topsoil at the six burnt and one unburnt study

sites are presented in Table 4. Immediately (IAF) and four months (4MAF) after the fire pH, EC and OM content were significantly higher in the burnt than unburnt eucalypt soils, with differences in values amounting to a factor 1.7, 3.4 and 1.6, respectively. Eight (8MAF) and fifteen (15MAF) months after the fire, however, these differences had become noticeably smaller or even inexistent. Accordingly, significantly ($p< 0.05$) higher values in the burnt than unburnt eucalypt soils were limited to the pH at sites BE1 and BE3, and to the OM content at the BE1 site. pH, EC and OM content did not differ significantly ($p> 0.05$) between the burnt soils of the eucalypt plantations and the burnt soil of the pine plantation.

The unburnt eucalypt soil did not reveal significant differences ($p> 0.05$) (in either pH, EC or OM content among the four sampling occasions. By contrast, the burnt soil under both eucalypt and pine did, with significantly ($p< 0.05$; Table 4) higher values of pH, EC and OM content immediately after the fire (IAF) than afterwards and, especially, eight and fifteen months after the fire (8MAF and 15MAF). In general, values of pH and OM content in soil burnt four months earlier (4MAF) were similar to or only slightly lower than to those in soils burnt less than one month earlier (IAF), while they had dropped up to 30 and 35 %, respectively, by the two subsequent sampling occasions (8MAF and 15MAF). EC values in burnt soils decreased most between the first and second sampling occasion (IAF and 4MAF), with up to 54 %, and did not differ between the last two sampling occasions (8MAF and 15MAF).

The aluminium (Al) contents at the seven study sites are also summarized in Table 4. The Al values ranged from 1.9 to 3.5 % in the burnt and unburnt eucalypt soils and from 2.1 to 3.6 % in the burnt pine soils. Furthermore, no significant ($p>0.05$) differences in Al concentrations were found between: (1) unburnt and burnt eucalypt plantations; (2) burnt soils of the two forest types (eucalypt *vs.* pine) and (3) the four sampling occasions (IAF, 4MAF, 8MAF, 15MAF).

Table 4 - pH, electric conductivity (EC, µScm^{-1}), organic matter (OM, %) and Aluminium (Al, %) in unburnt eucalypt and burnt eucalypt (UBE, BE1, BE2, BE3) and pine soils (BP1, BP2, BP3) at Ermida area. Samples were collected immediately after fire (IAF), 4 months after fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF). Mean values ± standard deviation are given. *Asterisks* indicate significantly differences between unburnt and burnt eucalypt samples. Different letters denote statistically significant differences ($p<0.05$) between sites during the same sampling period and different symbols between sampling periods at the individuals' sites. nd - not determined.

Site	Survey	pH	EC (µScm^{-1})	OM (%)	Al (%)
UBE	IAF	4.0 ± 0.02 ∇	120 ± 7.1 ∇	16 ± 1.3 ∇	2.0 ± 0.04 ∇
	4MAF	3.9 ± 0.13 ∇	125 ± 7.1 ∇	16 ± 1.2 ∇	2.1 ± 0.20 ∇
	8MAF	4.0 ± 0.15 ∇	128 ± 3.5 ∇	17 ± 1.2 ∇	2.0 ± 0.17 ∇
	15MAF	4.0 ± 0.03 ∇	113 ±3.5 ∇	16 ± 0.9 ∇	1.9 ± 0.24 ∇
BE1	IAF	6.4 ± 0.14 ∗a∇	363 ±10.6 ∗ac∇	24 ± 0.9 ∗ac∇	2.5 ± 0.161 a∇
	4MAF	6.0 ± 0.03 ∗ a∇◊	220 ± 14.1 ∗a◊	23 ± 1.5 ∗a∇□	2.6 ± 0.31 a∇
	8MAF	5.6 ± 0.15 ∗ a◊	130 ± 7.1 a□	21 ± 0.9 ∗a◊□	2.4 ± 0.44 a∇
	15MAF	4.4 ± 0.09 ∗ a□	123 ± 3.5 a□	19 ± 0.8 ∗a◊	2.3 ± 0.17 a∇
BE2	IAF	6.0 ± 0.11 ∗b∇	290 ± 14.1 ∗b∇	26 ± 1.0 ∗a∇	2.5 ± 0.29 a∇
	4MAF	4.8 ± 0.06 ∗b◊	173 ± 3.5 ∗b◊	26 ± 1.4 ∗b∇	2.9 ± 0.12 a∇
	8MAF	4.5 ± 0.14 b◊□	113 ± 3.5 a□	18 ± 1.3 bc◊	2.4 ± 0.40 a∇
	15MAF	4.2 ± 0.02 a□	100 ± 7.1 a□	17 ± 1.0 b◊	2.3 ± 0.35 a∇
BE3	IAF	6.7 ± 0.10 ∗a∇	403 ± 3.5 ∗a∇	15 ± 1.1 b∇	2.8 ± 0.55 a∇
	4MAF	6.5 ± 0.04 ∗c∇	368 ± 10.6 ∗c◊	15 ± 0.8 c∇	2.7 ± 0.38 a∇
	8MAF	5.8 ± 0.06 ∗a◊	168 ± 10.6 b□	16 ± 1.9 cd∇	3.5 ± 0.28 a∇
	15MAF	nd	nd	nd	nd
BP1	IAF	5.8 ± 0.02 b∇	335 ± 21.2 c∇	22 ± 1.0 c∇	3.2 ± 0.11 a∇
	4MAF	5.5 ± 0.06 d∇	155 ± 14.1 b◊	21 ± 1.3 d∇□	3.0 ± 0.08 a∇
	8MAF	4.5 ± 0.03 bc◊	123 ± 3.5 a□	19 ± 1.6 ab◊□	2.8 ± 0.54 a∇
	15MAF	4.1 ± 0.06 a◊	103 ± 3.5 a□	18 ± 0.9 ab◊	2.4 ± 0.35 a∇
BP2	IAF	5.9 ± 0.08 b∇	393 ± 10.6 a∇	15 ± 2.3 b∇	3.6 ± 0.17 a∇
	4MAF	5.7 ± 0.05 a∇	310 ± 7.1 d◊	15 ± 1.2 c∇	3.4 ± 0.23 a∇
	8MAF	5.2 ± 0.08 a◊	163 ± 3.5 a□	15 ± 0.5 d∇	3.3 ± 0.02 a∇
	15MAF	4.3 ± 0.05 a□	103 ± 3.5 a□	15 ± 2.1 b∇	2.1 ± 0.43 a∇
BP3	IAF	5.1 ± 0.11 c∇	203 ± 3.5 b∇	19 ± 1.2 d∇	3.1 ± 0.19 a∇
	4MAF	4.6 ± 0.06 b∇◊	125 ± 7.1 b◊	19 ± 1.5 d∇	3.2 ± 0.03 a∇
	8MAF	4.1 ± 0.06 c◊	100 ± 7.1 b□	15 ± 0.7 cd◊	3.0 ± 0.26 a∇
	15MAF	nd	nd	nd	nd

3.3.2 Physicochemical characteristics of ashes

The pH, EC and OM values of the ashes from the six burnt study sites are summarized in Table 5. The pH values revealed a distinct pattern with time-since-fire than the EC values and OM contents. The ranges in pH tended to decrease gradually between the four subsequent sampling occasions from 6.9-8.0 (IAF) to 6.8-7.6 (4MAF) and 6.5-7.2 (8MAF) and, finally, 6.4-6.8 (15MAF), but differences between the four

sampling occasions were generally not significant. (p> 0.05). By contrast, EC and OM values were clearly and significantly highest in the ashes collected IAF (1350-1950 µScm^{-1} and 32-46 %, respectively) than afterwards (140-360 µScm^{-1} and 19-31 %). The largest decrease in both EC and OM content had occurred by the fourth month after the fire, with up to 11.4 and 1.9 times, respectively. Values had further decreased 8MAF but not significantly, and then had generally remained unchanged at the end of this study (15MAF). Significant (p<0.001) differences between IAF and the other three sampling occasions (4, 8 and 15MAF) events were detected for EC and OM.

The ashes did not reveal significant differences between the eucalypt plantations and the pine plantation in either pH, EC or OM content.

Table 5 – pH, electric conductivity (EC, µScm^{-1}) and organic matter (OM, %) eucalypt and pine ashes (BE1, BE2, BE3, BP1, BP2, BP3) at Ermida area. Samples were collected immediately after the fire (IAF), 4 months after fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF). Mean values ± standard deviation are given. Different letters denote statistically significant differences (p<0.05) between sites during the same sampling period and different symbols between sampling periods at the individuals' sites. nd - not determined.

Site	Survey	pH	EC (µScm^{-1})	OM (%)
BE1	IAF	7.2 ± 0.21 a∇	1950 ± 70.7 a∇	42 ± 6.5 a∇
	4MAF	6.8 ± 0.05 a∇	275 ± 35.4 ac◊	29 ± 6.3 a◊
	8MAF	6.6 ± 0.14 a∇	148 ± 24.7 a◊	23 ± 5.6 a◊
	15MAF	6.4 ± 0.16 a∇	110 ± 14.1 a◊	24 ± 4.0 a◊
BE2	IAF	6.9 ± 0.02 a∇	1050 ± 70.7 b∇	46 ± 5.5 a∇
	4MAF	6.8 ± 0.15 a∇◊	360 ± 14.1 c◊	31 ± 5.0 a◊
	8MAF	6.5 ± 0.11 a∇◊	210 ± 42.4 a◊□	24 ± 3.1 a◊
	15MAF	6.4 ± 0.14 a◊	115 ± 14.1 a□	24 ± 1.6 a◊
BE3	IAF	8.0 ± 0.18 b∇	1550 ± 212.1 ab∇	39 ± 6.8 a∇
	4MAF	7.6 ± 0.08 b◊	188 ± 3.5 ab◊	23 ± 0.5 a◊
	8MAF	6.8 ± 0.08 a□	165 ± 21.2 a◊	22 ± 1.5 a◊
	15MAF	nd	nd	nd
BP1	IAF	7.3 ± 0.11 a∇	1375 ± 176.7 ab∇	37 ± 4.2 a∇
	4MAF	6. 9 ± 0.16 a∇	235 ± 7.1 a◊	19 ± 2.9 a◊
	8MAF	7.2 ± 0.33 a∇	198 ± 3.5 a◊	17 ± 2.4 a◊
	15MAF	6.8 ± 0.11 a∇	213 ± 17.6 b◊	20 ± 1.2 a◊
BP2	IAF	7.9 ± 0.07 b∇	1600 ± 212.1 ab∇	32 ± 7.9 a∇
	4MAF	7.1 ± 0.13 a◊	140 ± 14.1 b◊	19 ± 1.8 a◊
	8MAF	6.7 ± 0.10 a◊	133 ± 10.6 a◊	16 ± 1.9 a◊
	15MAF	6.5 ± 0.09 a◊	115 ± 14.1 a◊	18 ± 3.0 a◊
BP3	IAF	7.1 ± 0.26 a∇	1350 ± 141.4 ab∇	39 ± 5.7 a∇
	4MAF	7.3 ± 0.13 a∇	325 ± 35.4 c◊	24 ± 2.1 a◊
	8MAF	nd	nd	nd
	15MAF	nd	nd	nd

3.3.3 Major and trace elements in burnt and unburnt soils

The concentrations of the major and trace elements (V, Mn, Co, Ni, Cu, Cd and Pb) in the soils of the seven study sites are summarized in Figure 13. In general, the levels of Mn, Cd, Pb, Ni and V immediately after the fire (IAF) were significantly higher (p<0.05) at the burnt eucalypt sites compared with those at the unburnt eucalypt site, with values differing up to 13.4, 3.4, 3.1, 1.8 and 1.7 times, respectively. Although Mn, Cd, Pb, Ni, and V continued to exhibited higher concentrations in the burnt than unburnt eucalypt soils at the other three sampling occasions (4MAF, 8MAF,15MAF), these differences were only statistically significant (p<0.001) at all three instances in the case of Pb, Ni and V. In contrast, the concentrations of Co and Cu generally did not differ significantly (p>0.05) between the burnt and unburnt eucalypt soils through the study period.

The elements concentrations in the unburnt eucalypt soil generally did not reveal marked temporal patterns and, accordingly, no statistical differences (p>0.05) between the four sampling occasions (Figure 13). The same was true for the Cu and Pb levels in the burnt soils of both the eucalypt and pine plantations. The Mn and Cd concentrations in the burnt soils revealed a distinct temporal pattern, with a 2-14 fold and 2-fold, respectively, significant decrease between the first (IAF) and the second (4MAF) sampling occasion (except for Cd in BE2, BE3 and BP3, that did not change significantly with time since fire). Manganese and Cd levels in burnt soils collected 8 and 15MAF did not differed significantly (p>0.05) than those sampled 4MAF. Still a different temporal patterns was observed for the V, Ni and Co concentrations in burnt soils, increasing significantly up to 67%, 70% and 71%, respectively, during the surveyed first 8 months (from IAF until 8MAF). Between 8 and 15MAF it was observed a decrease of those elements in burnt soils.

Significant differences between the burnt eucalypt soils and the burnt pine soils were limited to two elements, Co and Ni (Figure 13; Table 6). In both instances, concentrations were higher at the pine than eucalypt sites.

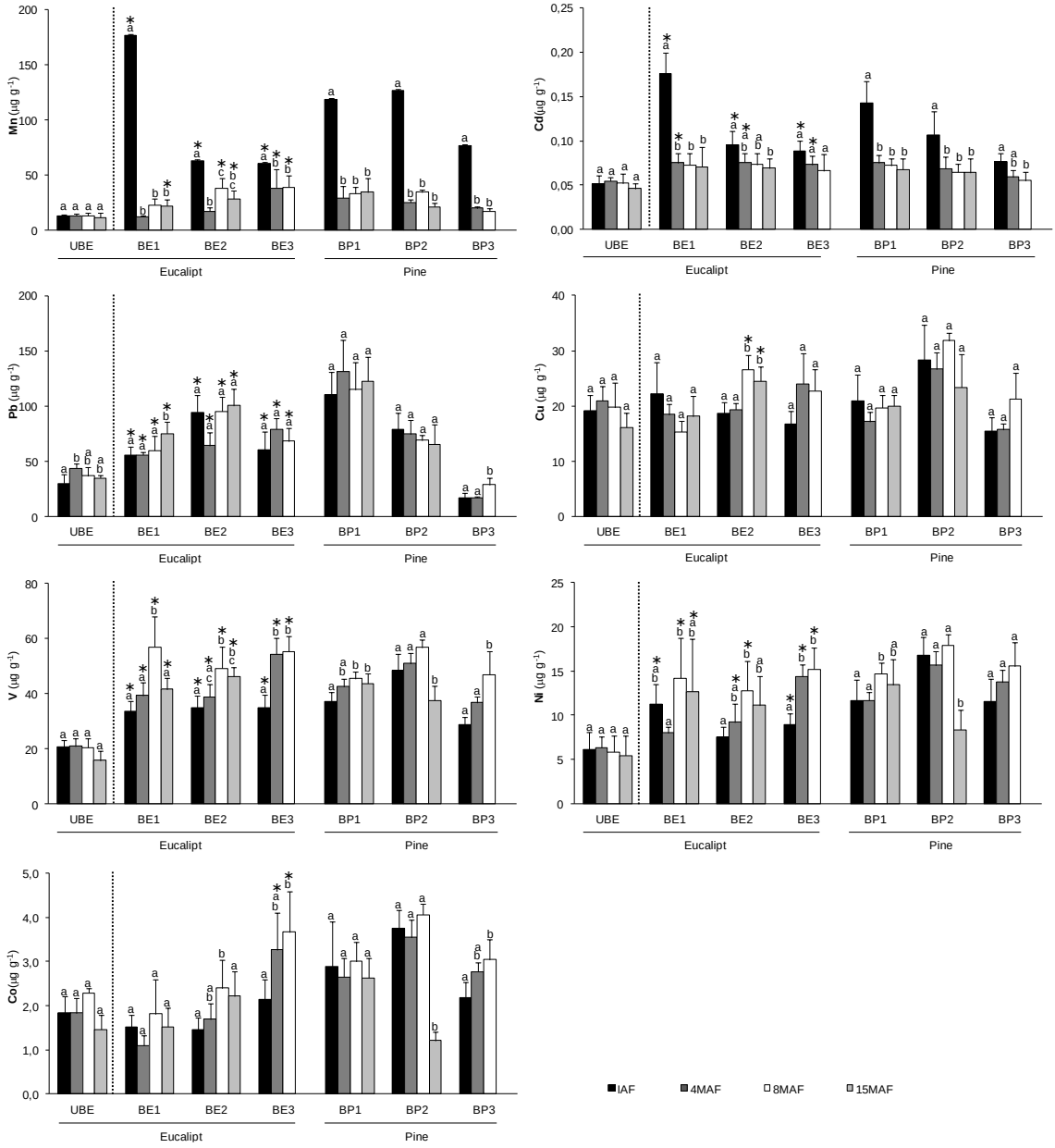

Figure 13 – Major and trace elements concentrations (µg g^{-1}) in soils collected at Ermida immediately after the fire (IAF), 4 months after the fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF). Mean and associated standard deviation are given. *Asterisks* indicate significantly differences for metals levels between unburnt (UBE) and burnt eucalypt sites at Ermida (BE1, BE2 and BE3). Different letters denote statistically significant differences (p<0.05) between sampling periods (for each site).

Table 6 – Two-way analysis of variance (ANOVA) testing the effect from the vegetation type (burnt eucalypt vs. burnt pine) and the survey influence (immediately, 4, 8 and 15 months after the fire) in the concentration of major and trace elements (Mn, Cd, Pb, Cu, V, Ni, Co) in burnt soils and ashes collected in Ermida.

Sample	Dependent variable	Source of variation	*df*	Mean square	F	*p*
Soil	Mn[a]	Site	1	0.202	1.328	0.252
		Survey	3	12.108	79.499	**< 0.001**
		Site x survey	3	0.246	1.615	0.191
	Cd[a]	Site	1	0.195	3.534	0.063
		Survey	3	1.622	29.458	**< 0.001**
		Site x survey	3	0.00282	0.0512	0.985
	Pb	Site	1	479.015	0.437	0.510
		Survey	3	2699.428	2.465	0.063
		Site x survey	3	270.866	0.247	0.863
	Cu	Site	1	87.719	2.781	0.098
		Survey	3	76.284	2.419	0.071
		Site x survey	3	7.578	0.240	0.868
	V	Site	1	29.386	0.568	0.453
		Survey	3	1208.192	23.342	**< 0.001**
		Site x survey	3	88.032	1.701	0.172
	Ni	Site	1	111.798	11.882	**< 0.001**
		Survey	3	86.959	9.242	**< 0.001**
		Site x survey	3	28.296	3.007	**0.034**
	Co	Site	1	14.059	21.617	**< 0.001**
		Survey	3	4.740	7.288	**< 0.001**
		Site x survey	3	1.275	1.961	0.125
Ash	Mn[a]	Site	1	0.0612	0.404	0.527
		Survey	3	10.213	67.362	**< 0.001**
		Site x survey	3	0.625	4.125	**0.008**
	Cd[a]	Site	1	2.739	67.837	**< 0.001**
		Survey	3	4.150	102.762	**< 0.001**
		Site x survey	3	0.00176	0.0436	0.988
	Pb	Site	1	2578.967	3.566	0.062
		Survey	3	362.780	0.502	0.682
		Site x survey	3	1773.843	2.453	0.068
	Cu	Site	1	0.376	0.00615	0.938
		Survey	3	1290.506	21.076	**< 0.001**
		Site x survey	3	78.564	1.283	0.285
	V[a]	Site	1	0.0816	2.358	0.128
		Survey	3	1.367	39.516	**< 0.001**
		Site x survey	3	0.268	7.745	**< 0.001**
	Ni	Site	1	116.902	6.684	**0.011**
		Survey	3	551.259	31.521	**< 0.001**
		Site x survey	3	31.756	1.816	0.150
	Co[a]	Site	1	0.949	18.694	**< 0.001**
		Survey	3	1.398	27.546	**< 0.001**
		Site x survey	3	0.581	11.438	**< 0.001**

df - degrees of freedom; F – F statistic; *p* – *p* value (probability level for significant effects: $p < 0.05$). Significant values ($p < 0.05$) are marked in bold
[a]Comparisons made with Ln transformed data

3.3.4 Major and trace elements in ashes

From the elements analysed in this study, Mn and Pb attained the highest and the second highest concentrations in the ashes, ranging from 67 to 598 μg g^{-1} and from 52 to 122 μg g^{-1}, respectively (Figure 14). In general, Co and Cd presented the lowest concentrations, with ranges of 1.8 to 4.8 μg g^{-1} 0.12 to 0.49 μg g^{-1}, respectively.

The concentrations of V, Mn, Ni, Cu and Cd had peaked in ashes collected IAF and then, declined sharply in the first 4 months ($p<0.005$). These values remained unchanged 8MAF and 15MAF, without statistical differences ($p>0.05$) between these three sampling periods, with the exception of V that revealed a further slightly decrease between the 4MAF and 8MAF. A similar pattern with time-since-fire was followed by Pb and Co, with a tendency to display higher concentrations in ashes collected IAF than 4MAF, but without statistical differences ($p<0.05$). This trend was not clear among the others surveys (4, 8 and 15MAF).

Like in the case of the burnt soils, Co and Ni had significantly ($p<0.005$) higher concentrations in the ashes of the pine plantations than of the eucalypt plantations (Table 6). The relation between the major and trace elements concentrations in the burnt soils and in the ashes are represented in Figure 15. The concentrations of Mn and Cd and, to a lesser extent, Co, Ni and Cu, in ashes were clearly higher than in burnt soils at all four sampling occasions, with differences of more than 70% and of 55%-75%, respectively, for all sampling periods. In contrast, Pb concentrations ranged identically in burnt soils and ashes, except in the case of BP3 that were higher in the ashes (75%). Vanadium concentrations revealed a distinct pattern, being characterised by higher values in ashes than in burnt soils for the first sampling occasion (IAF; 55%-70%), similar values for the second occasion (4MAF), and lower values (30%-45%) at the last two occasions (8MAF and 15MAF).

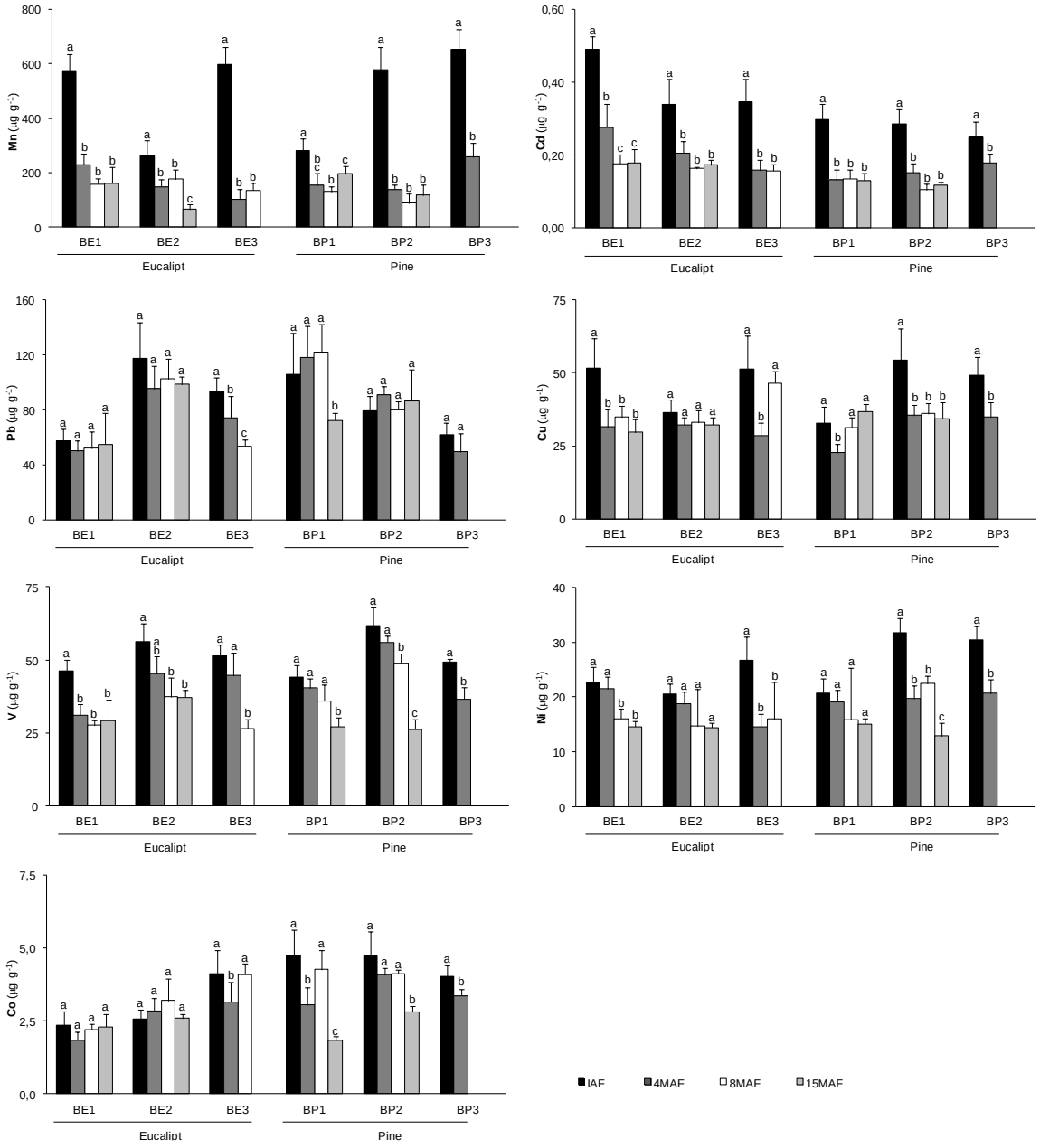

Figure 14 – Major and trace elements concentrations (µg g^{-1}) in ashes collected at Ermida immediately after the fire (IAF), 4 months after the fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF). Mean and associated standard deviation are given. Different letters denote statistically significant differences (p<0.05) between sampling periods (for each site).

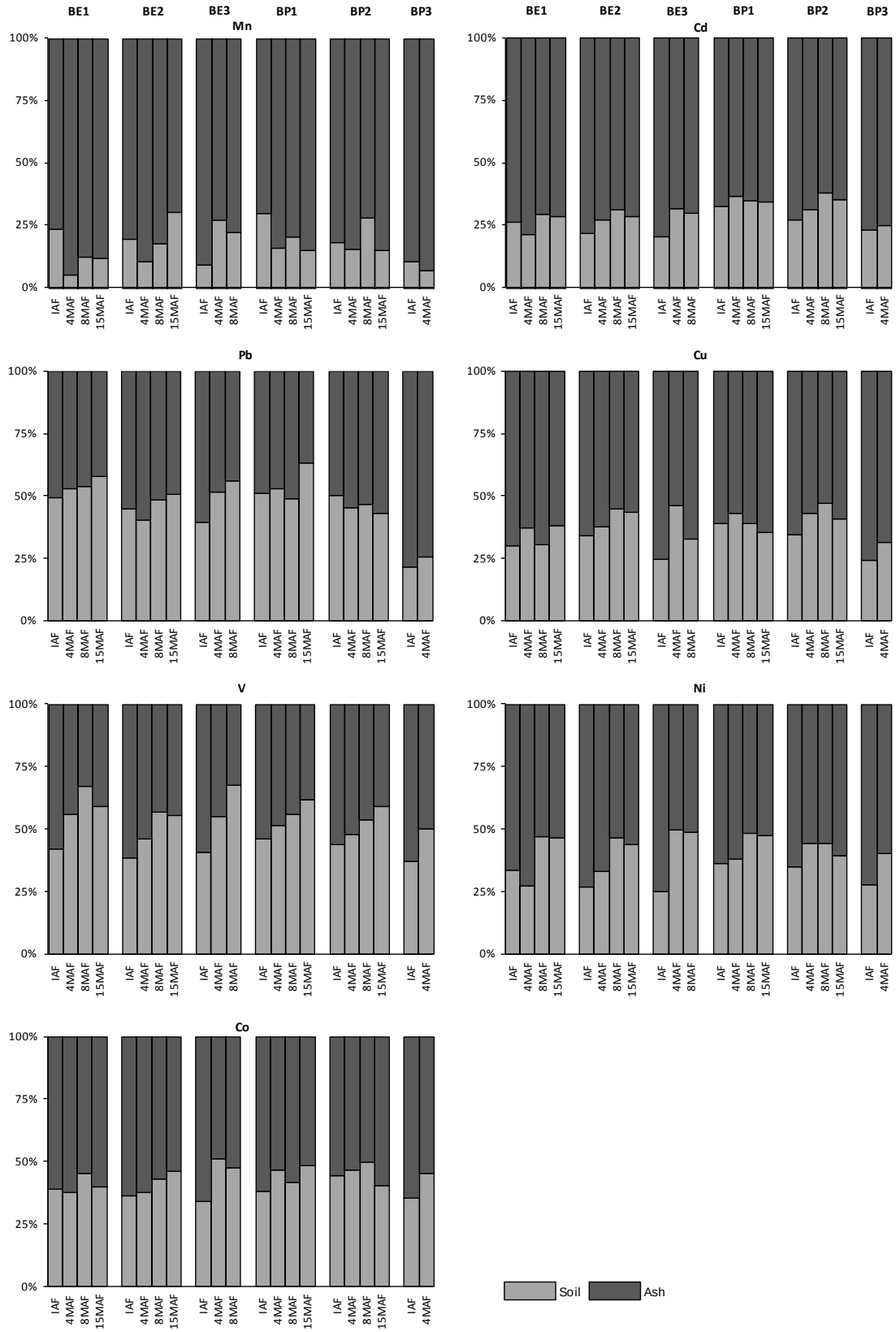

Figure 15 – Relation between major and trace elements concentrations in soils and ashes collected immediately after the fire (IAF), 4 months after the fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF) at burnt eucalypt and pine sites (BE1, BE2, BE3 and BP1, BP2, BP3, respectively).

3.3.5 Relationships of major and trace elements with soil and ash physicochemical characteristics

The relationship between major and trace elements concentrations in soils and ashes with pH, EC and OM contents were assessed using the Spearman rank correlations coefficients (Table 7). According to the values of Spearman coefficients, Mn and Cd contents in soils were moderately correlated to pH (r=0.615, p<0.001; r=0.635, p<0.001, respectively) and to EC (r=0.495, p<0.001; r=0.444, p<0.001, respectively). For the other elements those relationship were not significant (p>0.05), except for Pb and V, showing a poor correlation with pH (r= 0.355, p<0.05; r=0.287, p<0.05). Organic matter and major and trace elements were moderately correlated for Cd (r=0.440, p<0.001) and Co (r=-0.417, p<0.001), while the significance of this relationship was weaker for Cu (r=-0.282, p<0.01), Mn (r=-0.224, p<0.01) and Ni (r=0.179, p<0.05). In contrast, V and Pb were the only ones without a significant correlation (r=0.168, p>0.05; r=-0.092, p>0.05).

Manganese, Ni and Cd levels in ashes were moderately correlated to pH (r=0.582, p<0.001; r=0.671, p<0.001; r=0.462, p<0.01, respectively) and strongly correlated to EC (r=0.820, p<0.001; r=0.862, p<0.001; r=0.811, p<0.001, respectively). Manganese and Ni showed a moderate correlation with OM (r=0.662, p<0.001; r=0.519, p<0.001) while a strong correlation was found for Cd (r=0.818, p<0.001). Cobalt content showed a strong and moderate relationship with pH (r=0.813, p<0.001) and EC (r=0.497, p<0.01), respectively. Moderate correlations were found between V content and pH (r=0.664, p<0.001), EC (r=0.646, p<0.001) and OM (r=0.411, p<0.001), while for Cu level it was only found for EC (r=0.451, p<0.01) and OM (r=0.483, p<0.001). There were no relationships between Pb concentration and ash physicochemical properties.

Table 7 – Spearman rank correlations coefficients between major and trace elements concentrations (Mn, Cd, Pb, Cu, V, Ni, Co) and physicochemical parameters for soil and ash samples in Ermida.

	Survey	**Mn**	**Cd**	**Pb**	**Cu**	**V**	**Ni**	**Co**
	pH	0.615***	0.635***	0.355*	0.008	0.287*	0.221	0.113
SOIL	EC	0.495***	0.444***	0.085	0.027	0.026	0.112	0.171
	OM	0.224**	0.440***	0.168	-0.282**	-0.092	-0.179*	-0.417***
	pH	0.582***	0.462**	0.019	0.330	0.664***	0.671***	0.813***
ASH	EC	0.820***	0.811***	0.022	0.451**	0.646***	0.862***	0.497**
	OM	0.662***	0.818***	0.028	0.483***	0.411***	0.519***	0.216

Probability levels for significant effects: p < 0.001 (***); p < 0.01 (**);p < 0.05 (*).

3.3.6 Enrichment factors

The fire-related enrichment factors (EF_{WF}) for the major and trace elements in the burnt relative to unburnt eucalypt soils (BE1, BE2 and BE3 *vs.* UBE) are summarized in Table 8. These EF_{WF} generally were greater than 1.5, except in the cases of Cu and Co for which EF_{WF} averaged 1.0 and ranged from 0.9 to 1.2 and from 0.8 to 1.2, respectively. The maximum EF_{WF} was found for Mn, with an average of 7.6 and a range of 4.6-13.4, followed by Cd and Pb, with averages of 2.3 and ranges of 1.7-3.4 and 1.8-3.1, respectively. In the case of V (1.6-1.7) and Ni (1.2-1.8), the average values of the EF_{WF} were 1.6 and 1.5, respectively.

Table 8 - Enrichment factor wildfire related (EF_{WF}) of the major and trace elements in soil with respect to the background or reference (UBE) in soil collected immediately after the fire (IAF).

Survey	Mn	Cd	Pb	Cu	V	Ni	Co
BE1	13.4	3.4	1.8	1.2	1.6	1.8	0.8
BE2	4.8	1.9	3.1	1.0	1.7	1.2	0.8
BE3	4.6	1.7	2.0	0.9	1.7	1.5	1.2
Average	7.6	2.3	2.3	1.0	1.6	1.5	0.9

The rainfall—related enrichment factors (EF_R) of major and trace elements in soils and ashes at the second relative to the first sampling occasion immediately after the fire (4MAF/IAF) are given in Table 9. In the case of the unburnt eucalypt soil, the average remained one or slightly above (ranged from 1.0 to 1.1) for all the studied elements, except Pb for which it amounted to 1.5. In the case of the burnt soils, the EF_R were below one for Mn and Cd ranging from 0.1 to 0.6 and 0.4 to 0.8, respectively, as opposed to an EF_R above one for V with values ranging from 1.1 to 1.6. In contrast, the EF_R for Pb, Cu, Ni and Co, did not reveal a consistent pattern, varying from below to above 1 (between 0.7 and 1.6). In the case of ashes, the EF_R values for all the elements were, in general, below one. The average EF_R in ashes were found in the following order Pb (0.9; 0.8-1.2) > Co (0.8; 0.6-1.1) ≅ Ni (0.8; 0.5-1.0) ≅ V (0.8; 0.7-0.9) > Cu (0.7; 0.6-0.9) > Cd (0.6; 0.4-0.7) > Mn (0.4; 0.2-0.6).

Table 9 – Enrichment factor rainfall related (EF_R) of the major and trace elements in soil and ash from the unburnt (UBE) and burnt eucalypt (BE1, BE2, BE3) and pines sites (BP1, BP2, BP3) at Ermida, calculated by the quotient between samples collected 4 months after the fire (4MAF) and immediately after the fire (IAF).

	Survey	Mn	Cd	Pb	Cu	V	Ni	Co
SOIL	UBE	1.0	1.1	1.5	1.1	1.0	1.0	1.0
	BE1	0.1	0.4	1.0	0.8	1.2	0.7	0.7
	BE2	0.3	0.8	0.7	1.0	1.1	1.2	1.2
	BE3	0.6	0.8	1.3	1.4	1.6	1.6	1.5
	BP1	0.2	0.5	1.2	0.8	1.1	1.0	0.9
	BP2	0.2	0.6	0.9	0.9	1.1	0.9	0.9
	BP3	0.3	0.8	1.0	1.0	1.3	1.2	1.3
	Average	0.3	0.7	1.0	1.0	1.2	1.1	1.1
ASH	BE1	0.4	0.6	0.9	0.6	0.7	1.0	0.8
	BE2	0.6	0.6	0.8	0.9	0.8	0.9	1.1
	BE3	0.2	0.5	0.8	0.6	0.9	0.5	0.8
	BP1	0.5	0.4	1.1	0.7	0.9	0.9	0.6
	BP2	0.2	0.5	1.2	0.7	0.9	0.6	0.9
	BP3	0.4	0.7	0.8	0.7	0.7	0.7	0.8
	Average	0.4	0.6	0.9	0.7	0.8	0.8	0.8

3.4 Discussion

3.4.1 Wildfires effects on physicochemical properties of soils and ashes

Wildfires, through heating, combustion and ash deposition have been shown to alter the physical and chemical properties of the topsoils (Certini 2005; Verma and Jayakumar 2012). Accordingly, significant changes were observed in pH and EC in soils following the fire in Ermida. The present study revealed an increment of pH and EC in burnt eucalypt soils compared with unburnt eucalypt soil IAF, which in agreement with observations of several authors, who also reported that pH and EC values of burnt soils were greater than those of unburnt soils (Hernández et al., 1997; Quintana et al., 2007; Boerner et al., 2009). Soil pH and EC generally increased after forest fires, and then return to similar levels to those of the corresponding unburnt soils (Ulery et al., 1993, 1995; Granged et al., 2011a). Fire-induced changes in soil pH and EC are mainly

attributed to the combustion of organic matter and the associated deposition of ashes, releasing large quantities of inorganic ions (cations), oxides, hydroxides and carbonates in soils, as well as by decreasing organic acids produced during the biological oxidation of organic matter in the detritus (Ulery et al., 1993, 1995; Kutiel and Inbar, 1993; Verma and Jayakumar, 2012). In general, a similar pattern to pH and EC was found for OM (with the exception of BE3), where a steep increase (approximately 35%) was recorded for burnt eucalypt soil after the fire, in comparison with the unburnt, that was maintained throughout all post-fire studied period. Prior studies have attributed fire-induced increases in topsoil OM contents as resulting from new inputs as partly charred litter and leaves, forest necromass and residual ash (Gimeno-Garcia et al., 2000; Pardini et al., 2004; Knicker et al., 2005; Jovanovic et al., 2011; Faria et al., 2015).

The initial increases in pH, EC and OM content in burnt soils tended to decrease with time-since-fire. Reductions in soil pH and EC post-fire were also noticed by other authors (Kutiel and Inbar, 1993; Hernández et al., 1997; Kim et al., 2003; Granged et al., 2011a,b). Likewise, temporal decreases of OM content in burnt soils were observed by Kim et al. (2003). Worth stressing in this respect was that no such changes were observed at the unburnt eucalypt site. Such decreases with time-since-fire are usually attributed to the formation of new humus in combination with the leaching or lateral transport of ashes by post-fire rainfall, decreasing soluble hydroxide and carbonate concentrations in the remaining ashes (Ulery et al., 1993; Certini, 2005; Zavala et al., 2014).

Aluminium in soils is commonly used as a proxy for particles nature and size since it is a key factor of trace elements association (Windom et al., 1989). The low percentage of Al in the collected samples pointed out that soils consisted mainly of a mixture of sand with small proportion of fine particles, which were in line with the prevalence of the sand fraction in the topsoil (0-2 cm depth) at three other forest plantations on schist in the same study area (Machado et al., 2015). The overall lack of significant differences in the present Al concentrations (i.e. between burnt and unburnt, between sites as well as between sampling occasions) pointing out that the observed differences in major and trace elements concentrations were unrelated to difference in soil texture.

The high pH and EC values in the ashes immediately after the fire could be explained by the release of ions due to the mineralization of organic matter after fire as well as by high amounts of mineralized nutrients in the ashes (Notario del Pino et al., 2008; Úbeda et al., 2009; Pereira et al., 2011). Similarly high pH values in ash samples collected 1 and 2 months after the fire were observed by Kim et al., 2003 (pH = 7.24) and Goforth et al., 2005 (pH = 8.0), respectively. The present EC values were similar to those

in Pereira et al. (2011, 2012), after a prescribed fire, as well as to those in Úbeda et al, (2009), produced by laboratory heating. The extremely high OM contents of the ashes immediately after the wildfire suggested incomplete vegetation combustion, also in line with the ashes' dark color (Goforth et al., 2005; Bodi et al., 2014).

The ashes showed a noticeable decrease in pH and especially EC and OM content with time-since-fire, as the burnt soils did as well. In a more intense monitoring study, with observations at 20 to 60 day intervals, Ulery et al. (1993) also found ash pH to decrease markedly with time-since-fire, from 12.0 to 9.3.

Ashes presented higher pH, EC and OM values than the underlying soils, indicating that the ash deposition on the soil surface and the heat associated with forest fire affect the soil properties (Cass et al., 1984; Ulery et al., 1993; Kim et al., 2003; Goforth et al., 2005). Overall, changes of the physicochemical properties of burnt soils are deeply related with ashes formation.

3.4.2 Wildfires effects on major and trace elements in soils and ashes

As stated earlier, few prior studies examined the effects of wildfires on the levels of V, Mn, Co, Ni, Cu, Cd and Pb in forest soils. Five of these elements exhibited significantly higher concentrations in recently burnt than unburnt eucalypt soils, with Cu and Co being the exception. Fire-induced increases in soil concentrations have been reported earlier for V, Mn, Ni, Cd and Pb, (Parra et al., 1996; Plumlee et al., 2007; Bogacz et al., 2011; Costa et al., 2014). Manganese was the element that showed the strongest increase in burnt soils. Likewise, Parra et al. (1996) and Chambers and Attiwill (1994) observed marked increases in Mn concentrations of 100 and 279 % in burnt soils, respectively, with the latter figure relating to water-soluble manganese following laboratory heating at 400 °C. The Mn increases may have their origin in ashes (mainly in the form of oxides) from burnt vegetation (Parra et al., 1996), as manganese typically accumulates in tree leaves and especially needles of resinous species (Kabata-Pendias, 2011). From the correlation of Mn concentrations in plant tissue with those in soils, Auclair (1977) inferred that burning mobilized Mn in Canadian wetlands. Chambers and Attiwill (1994), however, attributed post-fire increase in soil Mn content to the heating-induced physiochemical breakdown of Mn complexed with soil organic matter. In the present study, the role of ashes as a source of Mn was supported by the markedly higher (up to 10 times) Mn levels in the ashes than in the underlying soils. Costa et al. (2014) reported Mn enrichments of ashes relatively to the underlying soils up to 5 times.

Similar to the present findings, others researchers had reported significant influence of fire in the concentrations of increases in burnt soil content of V, Ni, Cd and Pb (Morrell et al., 1986; Mandal and Sengupta, 2006; Plumlee et al., 2007; Bogacz et al. 2011; Jovanovic et al., 2011). The fire-induced increases in topsoil content observed for these four elements (V, Ni, Cd and Pb) could also derive from recently deposited ashes, given the higher concentrations in the ashes than underlying soils immediately after the wildfire (Mandal and Sengupta, 2006; Zhai et al., 2009). Generally, the accumulation of Cd and Pb in soils, are typically related to its sorption by organic matter, fixation by clay minerals, association with Mn oxides, Fe and Al (hydro) oxides (Riffaldi et al., 1976; Kaschl et al., 2002; Sipos et al., 2005; Itami and Yanai, 2006; Kabata-Pendias, 2011). Likewise, V and Ni distribution and mobility in soil seems to be related to soil organic matter clay minerals and Fe oxides (Kabata-Pendias, 2011).

Contrary to the other five elements, Co and Cu did not reveal significantly higher concentrations in burnt than unburnt topsoil but, like these elements, revealed clearly higher concentration in the recently deposited ashes than in the underlying soils. Even so, these findings were consistent with other study on Co and Cu contents in recently burnt areas (Costa et al., 2014).

Most of the analysed elements revealed comparable concentrations in the burnt soils under the two forest types, suggesting similar affinity to accumulate these elements. The two exceptions were Co and Ni, with significantly higher concentrations under pine than under eucalypt. Interestingly, the opposite was true for Hg concentrations in the same study area (Campos et al., 2015). Nevertheless, further studies are required to confirm whether pines, in comparison to eucalypts, create conditions that favour Co and Ni retention in soil.

Soil physicochemical parameters such as pH, EC and OM content are considered to play a key role in the mobilization and retention of major and trace elements in soils (Puls et al., 1991, Chlopecka et al., 1996; Mehes-Smith et al., 2013). In this study, however, no clear relationship was found between soil organic matter content and major and trace elements in soil (except for Co and Cd). Hernandez et al. (2003) and Wang et al. (2010) also found poor relations of major and trace elements concentrations with OM content, possibly due to the fact that for OM such kind of relationships did not assess the relevant reactive fraction of OM (Harter, 1983; McBride et al., 1997). In turn, soil pH that has a strong influence on the adsorption, retention and movement of these elements, showed a pattern that it was not straightforward for all the studied elements, since correlations were only found for Mn and Cd. Likewise, Wang et al. (2010) found generally poor relationships of major and trace element concentrations in soil with pH.

Nonetheless, the opposite was found by Hernandez et al., (2003). The results of the correlation analysis showed a large variety and complexity in relationship between the different physicochemical parameters and the analysed elements in soils.

The wildfires ashes studied revealed noticeable differences in the concentrations of the seven analysed elements, with the highest concentrations being those of Mn and the lowest those of Cd. The present results agreed well with other data for a variety of ashes, including ashes from wildfires, prescribed fires, laboratory heating experiments and wood combustion boilers. The values for all seven elements in the ashes collected immediately after the wildfire were within their typical ranges in ashes from wood, bark and leaves/needles (Mn: 40-17000 μg g^{-1}; Pb: 4-350 μg g^{-1}; V: 10-120 μg g^{-1}; Cu: 4-400 μg g^{-1}; Ni: 2-250 μg g^{-1}; Co: 1-20 μg g^{-1}; Cd: 0.1-25 μg g^{-1}) (Khanna et al., 1994; Someshwar, 1996; Pitman, 2006; Plumlee et al., 2007; Gabet et Bookter, 2011; Viana et al., 2012; Bodí et al., 2014; Costa et al., 2014; Santín et al., 2015). These broad ranges reflect the typically very heterogeneous nature of ashes, which, in turn, reflect a variety of factors such as plant species and parts (bark, timber and needles), the admixture of other flammable materials, soil type and climate, as well as combustion process (Someshwar, 1996; Bodí et al., 2014).

Unlike the soils, the ashes generally revealed significant correlations of major and trace element concentrations with physicochemical properties of pH, EC and OM, with correlation coefficients exceeding 0.45. It is well-known that the extractability of most elements in solid samples is greatly dependent of the acidity of the system. Lowering the pH of the extraction increases the exchange. In others words, the higher the ash pH and the more likely it will be that the elements remains immobilised in the ash (Evans et al., 1995). The same is to be expected for OM content, as the studied elements tend to become absorbed to OM-rich surfaces (Teixeira et al., 2010).

Forest type played a similar role in the concentrations of the analysed elements in the ashes than in the burnt topsoils, with significantly higher values of Co and Ni at the pine than eucalypt sites. Assuming that most of the collected ashes derived from the combustion of above ground parts of pine and eucalypt, it seems that pine accumulates more Co and Ni, than eucalypt. Like in the case of burnt soils, Hg also revealed consistent differences between the two forest types in ashes (Campos et al., 2015).

Overall, the levels of almost all the elements studied were higher in burnt soils than in unburnt soils suggesting that wildfires can act as a source of contamination. In fact, it can be stressed that ash deposited after the fire could add significant amounts of Mn, Pb, V, Ni and Cd to burnt soils. The role of ash, acting as an important agent influencing the levels of major and trace elements in burnt soils, is also supported by the higher

levels of those elements found in the ash samples comparatively to the underlying soils. This evidence is also reinforced by the data showing that the order of concentrations in soils (Cd<Co<Ni<Cu<V<Pb<Mn) is similar to the one found in ashes.

3.4.3 Temporal variation of major and trace elements levels in soils and ashes

Wildfires typically produce a strong hydrological and erosion response during the initial post-fire period which then gradually diminishes with time to pre-fire background levels (Cannon et al., 2001; Shakesby, 2011; Moody et al., 2013). Also in the present study area, overland flow and the associated losses of sediments, nutrients and cations were found to show marked peaks during the first major rainfall events following the fire and to be strongly related to rainfall amounts and/or intensities (Machado et al., 2015; Prats et al., 2014, in press; Ferreira et al., in press). These peaks in runoff and erosion could explain that the topsoil concentrations of the majority of major and trace elements studied here decreased most between the first and second sampling occasion, i.e. during the first four months after the fire. Worth mentioning in this respect is still that in the study region these initial post-fire sediment losses typically involve large fractions of organic matter (e.g. Malvar et al., 2011, 2015; Prats et al., 2012, 2014). The observed decreases in major and trace elements could thus have involved losses by overland flow in dissolved and/or particulate form, especially also attached to soil and ash organic matter particles as well as leaching to deeper soil layers. Additionally, their mobility, the nature of its association with organic matter, oxides and with other soluble species, soil ability to release the metal from the solid phase as well as soil structure and profile development could also have contributed to the observed initial decreased in the major and trace elements t (De Matos et al., 2001; Businelli et al., 2009; Violante et al., 2010).

Our study revealed three different patterns in the behaviour of the studied elements in burnt soil, with time: a) Mn and Cd; b) V, Co and Ni; and c) Cu and Pb. The abrupt decreased observed in Mn and Cd concentrations in burnt soils after the first heavy rainfall event, could be explained by their greater solubility/mobility and easier desorption by ion exchange procedures compared to the others elements (Harrison et al., 1981; Hem, 1989; Ingwersen et al., 2000). Costa et al. (2014) reported a decrease in Mn concentration in burnt soils collected about one year after the fire and an increase in surface water within and downstream of the burnt area 8 and 12 months after the fire. The mobility of Cd was demonstrated by Pagotto et al. (2001) through its sequential extraction from road-side soils samples. A plausible explanation for the increased levels of V, Co and Ni 4 and 8MAF is related to the fact that they are less soluble and mobile

than Mn and Cd and persist in the soil. The EF_R for these elements were slightly above one, which may indicate the transference of V, Co and Ni from ashes to soils. In contrast with the other studied elements, Cu and Pb tend to persist in soil in similar levels 15MAF, probably in the form of oxides, hydroxides and carbonates, which enhanced their accumulation in surface soils, limiting their capacity to be leached for groundwaters (Harrison et al., 1981; Pagotto et al., 2001; Intawongse and Dean, 2006). Cupper and Pb are more difficult to mobilized, being Pb one of the least mobile trace elements in soil with a long retention time (Heinrichs et al., 1980; Harrison et al., 1981). Unlike in this study, Costa et al., (2014) found that the Cu level in burnt soils tended to increase by the first year after the fire. Pagotto et al. (2001) used simple and sequential extractions, with different reagents to point-out that Cu and Pb are very immobile in soils and very unresponsive to variations of salinity and pH. Only high acidity or complexation conditions can mobilize them in appreciable quantities.

Although beyond the scope of the present study, microbial activity could have played an important role in the temporal patterns of major and trace elements reported here.. The immobilization of the analysed elements could be affected by their sorption to microbial biomass and exopolymers, their ingestion and intracellular sequestration, and their precipitation in the form of organic and inorganic compounds such as oxalates and sulphates (Geoffrey, 2004; Alkorta et al., 2004).

In ashes, the temporal decrease in the concentrations of all the studied elements, after the first's rainfall events (4MAF), was more consistent and more pronounced than in soils. These decreases were accompanied by marked reductions in the ash OM contents, possibly further enhancing the elements mobility. The differences between the elements' decreases in ash concentrations agreed well with the differences in their mobility discussed in the previous paragraph. For example, the largest decreases were those in Mn and Cd, while the smallest decrease was that in Pb.

3.4.4 Insights on environmental implications of wildfires

The current study pointed out that wildfires can mobilize major and trace elements to the environment, acting thus as a potential source of environmental contamination, in line with other studies (Plumlee et al., 2007; Smith et al., 2011; Costa et al., 2014; Silva et al., 2015; Campos et al., 2015; Santín et al:, 2015). Additionally, this study also suggested that heavy rainfall during the initial post-fire period can play an important role in the mobilization of these elements in the environment. Such a sequence of extreme

events may lead to important diffuse inputs of contaminants to rivers, groundwater and estuaries (Burke et al., 2010; Smith et al., 2011; Costa et al., 2014).

The wildfire-related enrichment factor (EF) used in this study proved a useful indicator to compare the degree of contamination by the different elements. EF values close to unity (0.5-1.5) indicate that an element is from crustal origin or due to natural processes; those less than 0.5 suggest a possible mobilization or depletion of metals; whereas values higher than 1.5 suggest an anthropogenic origin (Szefer et al., 1996; Feng et al, 2004; Bhuiyan et al., 2010; Singh et al., 2010). Furthermore, in the case of metals, values smaller than 2 indicate no to minimal contamination, (Sutherland, 2000). This would imply that soil contamination immediately after the fire was non-existent or minimal in the case of four of the elements analysed here (Table 8) - i.e. V, Ni, Co and Cu ($EFw_{WF}<2$) - but relevant in the cases of Mn, Cd and Pb ($EF_{WF}>2$). The soil contamination risk was also assessed by comparing the measured values with the Dutch soil quality standards for contamination (MHSPE, 2000), since these contamination intervention and target values have not yet been established in Portugal. Only in the case of V and Pb (Table 10), some of the measured concentrations were above the target values; however, all were below the intervention values.

The observed changes in soil chemical properties could affect soil functioning through their impacts on the composition and activity of microbial communities. Accumulation of major and trace elements in forest soils has been found provoke toxic responses in soils microorganisms (Certini, 2005; Hart et al., 2005), including by reducing microbial biomass (Hartmann et al., 2005) and by decreasing carbon mineralization and disturbing enzymatic activities (Frey et al., 2006). Metal stress has furthermore been found to change the structure and diversity of microbial communities (Hartmann et al., 2005; Frey et al., 2006). Overall, increased wildfire frequency combined with intensified post-fire rainfall are viewed as important threats to soil productivity and ecosystem resilience in the long term, through their implications in terms of not just soil (fertility) losses by erosion but also decline in microbial mineralising activities (Hernández et al., 1997; Pardini et al., 2004; Guénon et al., 2013; Goberna et al., 2012; Muñoz-Rojas et al., 2016).

The risks of contamination posed by wildfires are not limited to the in-situ, burnt soils, but include surface and groundwater bodies within and downstream of a burnt area (Lasanta and Cerdà, 2005; Shakesby and Doerr, 2006; Smith et al., 2011). Worth stressing in this respect is that some of the present elements are toxic in very low concentrations and have a strong tendency to persist and accumulate in the environment. Although the export of the present elements by overland flow was beyond

the scope of this study, the observed decreases in ash and soil concentrations combined with the relevant erosion rates measured at other sites within the same burnt area did suggest that off-site contamination risks should be a topic for future research in the study region. In other regions, contaminant loadings from burnt areas were found to constitute a substantial contribution to the total contaminant load in downstream areas, especially during the first few years after the fire. (Gallaher and Knoch, 2004; Stein at al., 2012; Costa et al., 2014).

Table 10 - Dutch target values and soil remediation intervention values (mg kg^{-1}) in soils/sediments for trace elements.

Element	Dutch values		Range in this study
	Target	Intervention	
Mn	nd	nd	13-177
Cd	0.8	12	0.06-0.18
Pb	85	530	17-132
Cu	36	190	15-32
V	42	250[a]	29-57
Ni	35	210	8-18
Co	9	240	1.1-4.1

[a] -For the Vanadium (V), the intervention value is substituted for indicative levels for serious soil contamination; not determined.

3.5 Conclusion

Understanding the dynamic of major and trace elements on the environment is essential for ecosystem management and risk assessment of environmental contamination and sustainability. Current findings clearly pointed out the role of wildfires in the mobilization of major and trace elements in the environment:

- The levels of major and trace elements (with the exception of Cu and Co) were higher in burnt soils than in unburnt soils, suggesting an incorporation of these elements in burnt soils during or after the fire;
- Ashes were consistently enriched in all seven major and trace elements compared to the underlying topsoils, indicating that the deposition of wildfire ashes on the soil surface can contribute to increases in topsoil concentrations following the wildfire;

- The two forest types did not differ in topsoil or ash concentrations of four out of seven major and trace elements, all three exceptions attaining higher values in the pine soils and ashes (Co and Ni);
- The major and trace element concentrations in both soils and ashes generally decreased with time-since-fire and most notably so over the first four months after the fire, which could be explained by the heavy rainfall and associated peaks in overland flow during this initial period of the window-of-disturbance and would then imply diffuse contamination of surface water and/or groundwater.

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Chapter IV

Effects of forest fires on the levels of polycyclic aromatic hydrocarbons in soils and ashes

4. Effects of forest fires on the levels of polycyclic aromatic hydrocarbons in soils and ashes

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants, which have been raising important health and environmental concerns due to their toxic, carcinogenic, mutagenic and teratogenic activity, as well as their environmental persistence and tendency for bioaccumulation. Although forest fires have been identified as an important source of PAHs, few studies have quantified PAH contents of ashes and topsoil in recently burnt areas. Therefore, this study analysed the contents of fifteen PAHs, identified as priority by the United States Environmental Protection Agency, in ash and soil samples collected immediately after a wildfire in two areas in north-central Portugal (Ermida and S. Pedro do Sul) as well as 4, 8 and 15 months later in Ermida. The role of forest type was determined by sampling burnt eucalypt and pine plantations in both areas. For Ermida a long-unburnt eucalypt plantation was also sampled. The PAHs were analysed by gas chromatography-mass spectrometry (GC-MS), after a pressurized liquid extraction (PLE). Immediately after fire the concentrations of PAHs in burnt soils were approximately 4 times higher than in the corresponding unburnt soil samples. The soils and ashes were characterized by higher levels of PAHs with three to four rings, however the homologue profile differed between the unburnt and burnt soils. Nevertheless, with time since fire, these levels decreased considerably to those of the unburnt soil and the composition profiles became similar to those in unburnt soils, in which no temporal differences were recorded. The levels of PAHs in ashes were consistently enriched in PAHs relatively to corresponding soils, suggesting that soil were contaminated by ash-derived PAHs. Our results clearly suggest that PAHs produced by the forest fires seems to be absorbed by soil and ash, being ash one of the main agent influencing the concentration of PAHs in the soil. The composition profile and the values of PAHs isomeric diagnostic ratios indicate that forest soil PAHs

were mainly originated from biomass burning. Moreover, the decreased in PAHs levels over time, mainly due to the leaching and washout of the ash, may indicate that PAHs can be transport to groundwater systems and downstream by soil/ash runoff. This study highlighted the importance of wildfire and subsequent rainfall in the mobilisation and transport of PAHs in the environment. Further studies are important to assess the impact of PAHs in aquatic systems downstream to burnt areas.

Keywords: Wildfire, PAH, Soil, Ash, Portugal

4.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, which have caused major concerns in terms of public health and environment due to their, toxic, carcinogenic, mutagenic and teratogenic activity (IARC, 1991; ATSDR, 1995; European Commission, 2006; Wang et al., 2013), as well as their environmental persistence and tendency for bioaccumulation (Delistray, 1997; Rappaport et al., 2004; Cachot et al., 2006). Several international organizations [World Health Organization (WHO), US environmental Protection Agency (USEPA), Agency of Toxic Substances and Disease Register (ATSDR), International Agency for Research on Cancer (IARC) and European Community] have classified some PAHs among the most hazardous and persistent organic pollutants, being listed as priority pollutant.

PAHs production can be due to natural processes such as volcanic activity and wildfires, as well as to anthropogenic activities such as combustion of fossil fuels and biomass, and coke production. Hence, as a result of distinct point and diffuse sources of pollution, as well as due to their intrinsic properties, PAHs can be found in several compartments, contaminating air, soil and water. In what concerns the terrestrial compartment, PAH concentrations and their distribution in soils not only depend on the increasing impacts of industry, traffic and domestic heating (Bucheli et al., 2004) but also on the molecular weight of the compound, the form in which they are transported through the air and proximity to the source of pollution (Pontevedra-Pombal et al., 2012). In general, PAH contents in soil decrease exponentially with increasing distances from a point source of pollution (Van Brummelen et al., 1996; Wilcke et al., 1996). Accumulation of PAHs in soils may lead to contamination of plants (Muller and Shann, 2006), and through the food chain, may cause direct or indirect exposure to humans. Furthermore, evaporation of PAHs and their leaching and migration may lead to contamination of the atmosphere and groundwater, respectively (Srogi, 2007). Knowledge of PAH contents in soils are therefore a prerequisite for avoiding risks to environment and human health (Srogi, 2007).

PAH contents in soils and their possible sources have been less well studied for forest soils (Bucheli et al., 2004; Xiao et al., 2014) than for urban and agricultural soils (e.g. Bradley et al., 1994; Tay and Biney, 2013; Wang et al., 2013). Nonetheless, forest soils are widely regarded as an important long-term repository of PAHs, mainly because of the large interception areas of trees and by the high organic matter contents of forest soils (Wilcke et al., 1996; Srogi, 2007). In turn, forest fires have been identified as an important source of PAHs, with smoke from biomass burning revealing high PAH

concentrations (Freeman and Cattell, 1990; Radojevic, 2003; Ravindra et al., 2008). The type of biomass being burnt and characteristics of the fire itself such as fire intensity play a key role in the PAHs concentrations of the emitted smoke (Vergnoux et al., 2011). The effects of forest fires on PAH contents in soils, however, have been poorly studied (Garcia-Falcón et al., 2006; Vergnoux et al., 2011; Kim et al., 2011) and have not been studied before in Portugal, in spite wildfires have, on average, affected 144.000 ha per year in Portugal over the last decade (ICNF, 2013). Apparently, fire-produced PAHs are adsorbed by the ash layer deposited on the soil surface as well as by the organic matter in the topsoil, making them susceptible to post-fire erosion by wind and especially water as well as to leaching into the soil and, eventually, groundwater. PAH concentrations in recently burnt soils have been found to decrease gradually with time-since-fire but, at the same time, to maintain levels that pose a long-term threat to the environment (Olivella et al., 2006; Vergnoux et al., 2011).

The present study aimed to address the current research gap on fire effects on PAH contents of forest soils by quantifying the concentrations and profiles of PAHs of the ash layer and topsoil immediately after wildfire as well as their evolution with time-since-fire. This was done for two contrasting wildfires as well as for the two principal forest types in north-central Portugal – i.e. maritime pine and eucalypt plantations. Both forest types are well-known to be fire-prone (Moreira et al., 2013; Maia et al., 2014) and, on sloping terrain, to be susceptible to elevated risks of post-fire erosion (Malvar et al., 2011; Prats et al., 2013, 2014).

4.2 Materials and methods

4.2.1 Study area

Two study areas located in north-central Portugal: Ermida (40° 43’ 33” N, 8° 20’ 60’’ W, 170 m a.s.l., Aveiro district) and S. Pedro do Sul (40° 45’ 35’’ N, 8° 10’ 04’’ W, 370 m a.s.l., Viseu district) (Figure 16) were selected during the summer of 2010, as soon as possible after the occurrence of two wildfires. The wildfire of Ermida burnt almost 300 ha on 26 July 2010, whilst that of S. Pedro do Sul burnt over 5,000 ha on 6 of August 2010 (DUDF, 2011). The study areas were, at the time of the wildfires, predominantly covered by plantations of eucalypt (Eucalyptus globulus Labill.) and maritime pine (Pinus pinaster Ait.).

The climate of the two study areas can be characterised as humid meso-thermal, with wet winters and prolonged dry and warm summers (Köppen Csb; DRA, 2002). The

long-term mean annual temperature was in the range of 12-19 °C in the case of Ermida and in the range of 13-15 °C in the case of S. Pedro do Sul (SNIRH, 2013). Mean annual rainfall over the past three decades amounted to 1609 mm at Ermida and 1064 mm at S. Pedro do Sul (SNIRH, 2013). The soils of the two study areas were classified as predominantly humic Cambisols (Cardoso et al., 1971). The soils in Ermida, were principally developed over pre-Ordovician schists, whereas those in S. Pedro do Sul were typically derived from Hercynian granites (Ferreira, 1978).

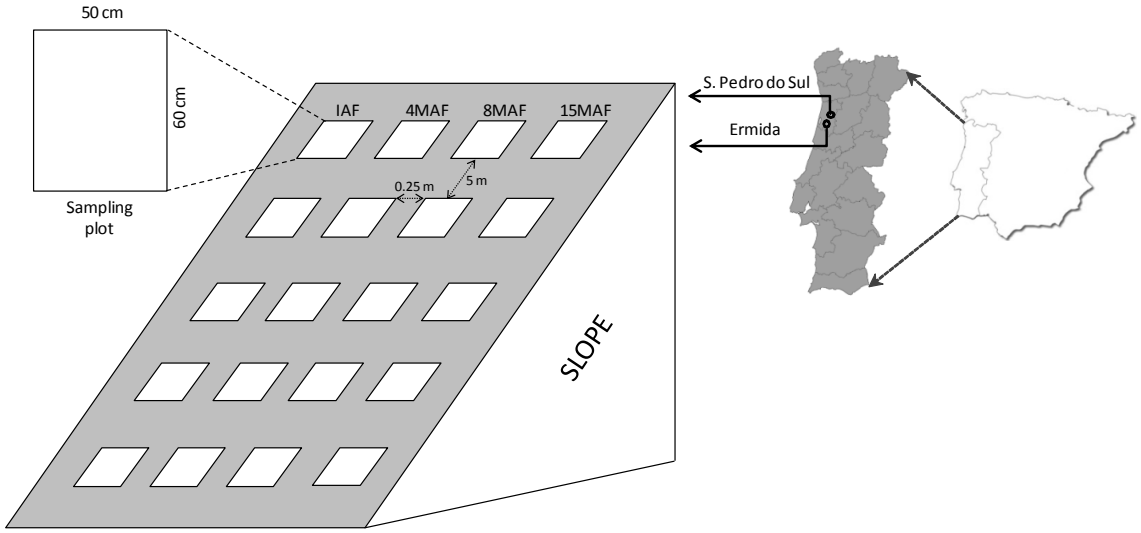

Figure 16 – Location of sampling areas (Ermida and S. Pedro do Sul) and design for soil and ash sampling.

4.2.2 Study sites and sampling design

In both burnt areas, one representative eucalypt plantation (BE) and one representative maritime pine plantation (BP) were selected. The two plantations in the area of S. Pedro do Sul were selected to represent a high fire severity (sensu Keeley, 2009), whilst the two plantations in the area of Ermida were selected to represent a moderate fire severity. To this end, fire severity was assessed in the field following Shakesby and Doerr (2006), and as applied by several prior studies in the region such as Keizer et al. (2008) in eucalypt plantations and Maia et al. (2012) in pine plantations. This was done at five points along a transect covering the plantation from the base to the top of the slope section (Figure 16), recording at each point the degree of canopy consumption of the 10 nearest trees (partial or total), the degree of litter consumption (partial or total), and ash colour (black, grey or white). Long-unburnt plantations with

comparable environmental as well as stand characteristics were searched in the vicinities of the two burnt areas but could only be found in one instance, i.e. a long-unburnt eucalypt plantation in the Ermida area (UBE).

In each plantation, a transect with five equidistant points was laid out from the top to the bottom of the slope section to address spatial heterogeneity in ash and soil characteristics (Figure 16). At each transect point, the ashes within a plot of 50 cm by 60 cm were carefully collected excluding any litter and surface stones and avoiding mixing-in soil, after which the upper 2 cm of soil were sampled.

Sampling at Ermida was carried out at the following four occasions: immediately after the fire (IAF) and 4, 8 and 15 months after the fire (4MAF, 8MAF and 15MAF, respectively).

Over the entire study period, 1472 mm of rainfall were registered, i.e. slightly less than the long-term annual mean. While rainfall between the fire and the first sampling occasion was negligible (4.3 mm), rainfall between subsequent sampling occasions varied markedly from 461 mm (first-to-second) to 739 mm (second-to-third) and 272 mm (third-to-fourth). Worth mentioning is that the 4MAF sampling took place after a period of particularly intense rainfall, totalling 219 mm. Sampling at S. Pedro do Sul was done only immediately after the fire (IAF).

Immediately upon arrival in the laboratory, the ash and soil samples were air-dried, sieved (2-mm mesh), homogenised with a mortar and stored in aluminium bags at -18 °C until further analysis.

4.2.3 Analytical procedures

The organic matter content (OM) of the ash and soil samples was determined by loss on ignition at 550 ºC for 4 h (ISO 11465; Botelho da Costa, 2004).

The PAH contents of the ash and soil samples were analysed following the procedure described in Martins et al. (2012). Prior to the actual analysis, however, the unfrozen samples were dried at 40 ºC to minimize the loss of the volatile PAHs (Berset et al., 1999). Pressurized liquid extraction (PLE) was performed using an ASE (Accelerated Solvent Extraction) 200 system (Dionex, USA) equipped with 24 stainless steel extraction cells. The extraction cells were lined with cellulose filter paper, packed (from bottom to top) with 5 g of sample mixed with diatomaceous earth, spiked with 1 ml surrogate standards obtained from Supelco (Bellefonte, PA, USA) containing acenaphthene-d_{10} (0.408 µg ml^{-1}), phenanthrene- d_{10} (0.397 µg ml^{-1}), chrysene-d_{12} (0.397

µg ml^{-1}) and perylene-d_{12} (0.433 µg ml^{-1}) and topped with cellulose filter paper. Extractions were performed with a mixture of hexane:acetone (1:1, v:v) at 100 ºC and 1500 psi for 5 min, followed by a static extraction step, with one cycle (5 min) and a flush volume of 60% of the extraction cell volume. Organic extracts were then concentrated by a rotator evaporator rinsed with hexane. The extracts were evaporated under a gentle stream of N_2 to 2 ml and fractionated with silica:alumina (1:1) and sodium sulphate glass column. The first fraction, corresponding to aliphatic hydrocarbons, was eluted with 20 ml of n-hexane and not analysed. The second fraction, containing the PAHs compounds, was eluted with 30 ml of a hexane of n-hexane/dichloromethane (9:1, v:v) and 40 ml n-hexane/ dichloromethane (4:1, v:v), evaporated by a rotator evaporator and then concentrated to 0.5 ml under a gentle stream of N_2 for prior analysis. Analyses of PAHs were performed on a gas chromatography-mass spectrometry (GC-MS) system (Thermo® DSQ) equipped with a DB-5MS column (30 m, 0.25 mm ID, 0.25 µm film thickness; Argilent, USA). The temperature program of capillary column was as follow: 75 ºC initial, then increased to 140 ºC at a rate of 30 ºC min^{-1}, followed by another ramp step at 3 ºC min^{-1} to an isothermal hold at 270 ºC for 15 min. Injection was achieved by auto-sampler in splitless mode, at 280 ºC and, interface line and ion source temperature maintained at 220 ºC. Helium was used as carrier gas at a flow of 1.0 ml min^{-1}. The MS was operated under electron impact ionization conditions (70 eV electron energy) and data were obtained in the selected ion monitoring (SIM) mode. Relevant standards were run to check column performance, peak height and resolution, before analysis. With each set of samples to be analysed, a solvent blank, a standard mixture and a procedural blank were run in sequence to check for contamination, peak identification and quantification.

In total, 15 PAHs were analysed. They were the 15 PAHs designated as priority by the US Environmental Protection Agency (USEPA) – i.e. acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT) (three-ring compounds); fluoranthene (FLT), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR) (four-rings); benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(ah)anthracene (DBahA), (five-rings); indeno(1,2,3-cd)pyrene (IND) and benzo(g,h,i)perylene (BghiP) (six-rings). The identification of these 15 PAHs was based on the comparison of GC-retention times and mass spectra with the appropriate standards of these individual compounds. The concentrations of the individual PAH were estimated by means of the internal standard peaks area method, using the ion ratio (m/z) of a standard PAH solution SRM 2260a (NIST, USA) and a nine-point calibration curve for each compound. A procedural blank was included in every set

of extraction samples to control possible contamination during the analytical procedure. The detection limit was calculated with a signal-to-noise ratio of 3:1 in a blank sample (n=10) and varied within a narrow interval of 0.001 μg g^{-1}. Quality control was carried out by analysing certified material SRM 1941b (NIST, USA). Recovery of analysed PAHs in the certified material ranged from 80% to 118%. All PAH contents reported in this study are given in ng g^{-1} dw. The total levels of the 15 USEPA prioritized PAHs (excluding the naphthalene- NAP) were defined as ∑PAHs.

4.2.4 Data analysis

The Student's t test was used to assess whether the ash and soil samples of the unburnt and burnt eucalypt plantations were significantly different. For each study area, a two-way ANOVA was applied to infer the overall statistical significance of differences in the OM and PAHs contents of the ash and soil samples between the two forest types (eucalypt vs. pine) and among the four sampling occasions (IAF, 4MAF, 8MAF and 15MAF). Specific contrasts between these factors were analyzed ad posteriori using the Tukey multiple comparison test. The assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk test and the Levene median test, respectively. When these assumptions were not met, data were transformed as neperian logarithm (Ln). The Spearman correlation coefficient was computed and tested for significance to examine the relationships between PAHs and OM contents in the ash and soil samples. All statistical tests were carried out using a α of 0.05.

Molecular ratios, such as BaA/(BaA+CHR) and FLT/(FLT+PYR), among others, have been proven to be useful in PAHs source identification (Yunker et al., 2002; Sprovieri et al., 2007). Hence, and due to the fact that these two isomer ratios revealed the best separation between the study samples, they were used to identify the possible sources of PAHs in soils from burnt areas.

4.3 Results

4.3.1 Total soil PAHs contents

The total contents of the 15 USEPA prioritized PAHs of the collected soil samples were summarized in Figure 17. The wildfire appeared to have had a significant direct effect on the total PAH content of the eucalypt soils in Ermida, as the total content immediately after the fire was, on average, four times as high at the burnt than unburnt

site (148 vs. 36,5 ng g-1). However, this effect was short-lived as none of the ensuing three sampling occasions revealed significant differences in total PAHs content between the burnt and unburnt eucalypt plantations in Ermida (Figure 17 and Table 11). This lack of significant differences was first and foremost due to substantial decreases in the total PAHs content at the burnt eucalypt plantation during the first eight months after fire, exceeding 50 % in both instances. A conspicuous but non-significant variation in total PAHs content also occurred at the unburnt eucalypt plantation in Ermida, but with a different timing than at the burnt eucalypt plantation (at the fourth month after the fire).

The total PAHs content of the burnt pine soil in Ermida revealed a similar temporal pattern as the total PAHs content of burnt eucalypt soil in Ermida. For both forest types the total PAHs content decreased substantially during the first eight months after the fire (with -80 and -79 %, respectively) and then again increased markedly between month 8 and 15 (with +64 and +61 %, respectively); furthermore, only the first of these differences between subsequent sampling occasions was statistically significant. The actual total PAHs contents, however, were clearly higher for the burnt pine soil than for the burnt eucalypt soil in Ermida, with differences ranging from +52 to +64 % throughout the study period. Nonetheless, from these differences only that of the first sampling occasion was statistically significant (Figure 17 and Table 11). Also, in the S. Pedro do Sul area, the burnt pine soil had a significantly higher total PAHs content than the burnt eucalypt soil immediately after the fire (+30 %). The total PAHs contents of the S. Pedro do Sul sites were, on average, lower than those of the Ermida sites (eucalypt: -10 %; pine: –30 %) but the difference between the two study areas was not statistically significant for either forest type.

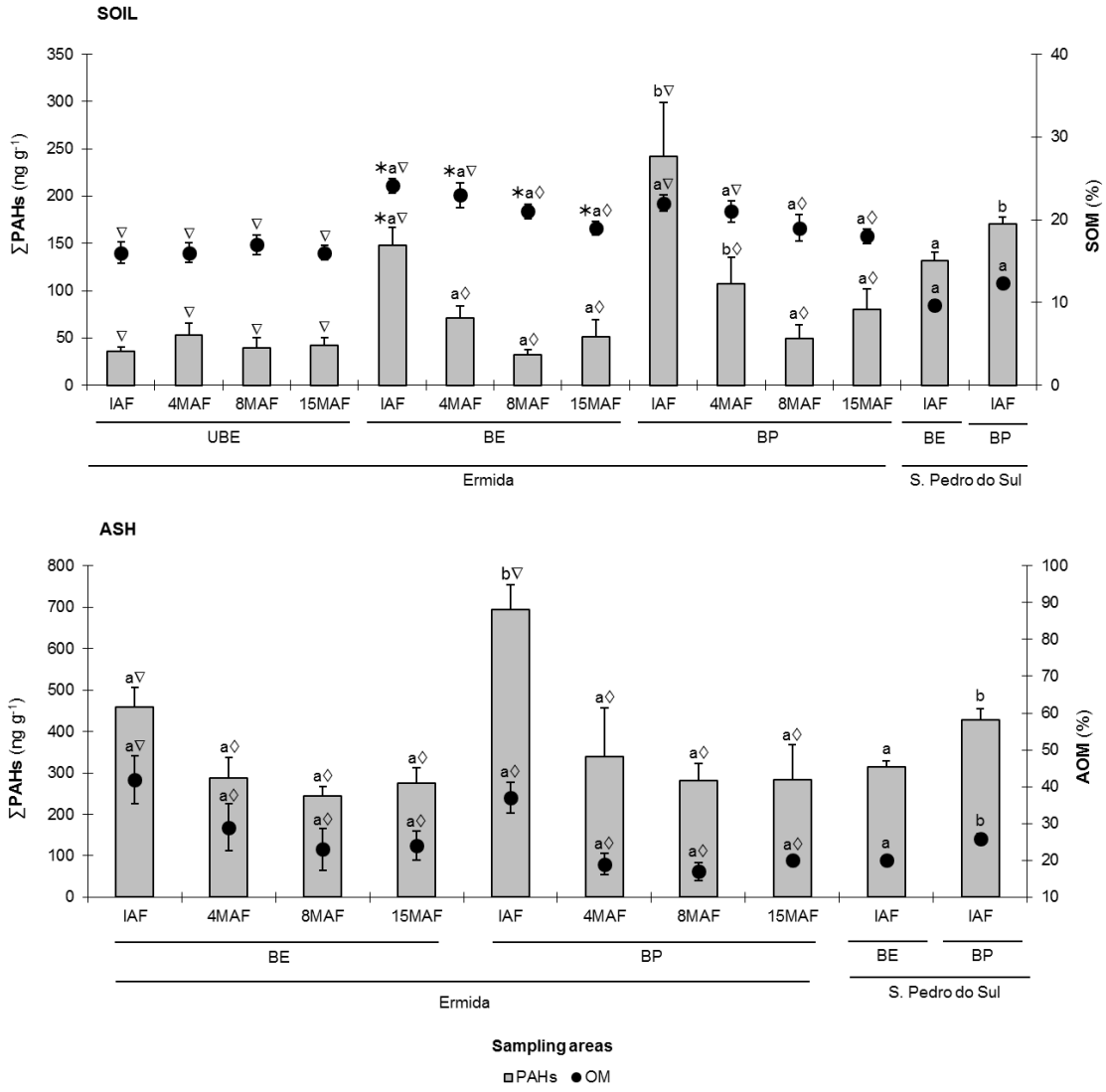

Figure 17 – Mean contents and standard deviations of ∑PAHs and organic matter of soil (SOM) and ash (AOM) samples collected in unburnt (UBE) and burnt (BE) eucalypt plantations and burnt pine plantations (BP) in Ermida and S. Pedro do Sul immediately and 4, 8, 15 months after fire (IAF, 4MAF, 8MAF and 15MAF, respectively). *Asterisks* indicate significant differences (p<0.05) in PAHs and OM contents between unburnt and burnt eucalypt soil in Ermida. Different letters denote statistically significant differences (p<0.05) in PAHs and OM contents between sites for each sampling period and different symbols between sampling periods at the individual sites (UBE, BE, BP), respectively.

Table 11 – Two-way analysis of variance (ANOVA) testing the effect from the vegetation type (burnt eucalypt vs. burnt pine) and the survey influence (immediately, 4, 8 and 15 months after fire) in the concentration of total PAHs (∑PAHs) and total content by number of aromatic rings (3, 4, 5 and 6 rings) in burnt soils and ashes collected in Ermida.

Sample	Dependent variable	Source of variation	*df*	Mean square	F	*p*
Soil	3 rings	Site	1	2702.813	10.095	0.006
		Survey	3	14976.057	55.599	<0.001
		Site x survey	3	370.599	1.384	0.284
	4 rings	Site	1	1750.521	21.390	<0.001
		Survey	3	1447.934	17.693	<0.001
		Site x survey	3	261.342	3.193	0.052
	5 rings	Site	1	51.091	6.826	0.019
		Survey	3	152.254	20.341	<0.001
		Site x survey	3	5.336	5.336	0.010
	6 rings	Site	1	29.989	10.960	0.006
		Survey	3	68.503	25.037	<0.001
		Site x survey	3	13.253	4.844	0.018
	∑PAHs	Site	1	10485.753	14.913	0.001
		Survey	3	27970.141	39.780	<0.001
		Site x survey	3	2767.483	2.514	0.095
Ash	3 rings	Site	1	21877.632	14.166	0.002
		Survey	3	94004.606	60.868	<0.001
		Site x survey	3	10433.699	6.756	0.004
	4 rings	Site	1	2217.101	6.474	0.022
		Survey	3	2392.527	6.996	0.003
		Site x survey	3	520.483	1.522	0.247
	5 rings	Site	1	8.568	0.367	0.553
		Survey	3	839.946	35.954	<0.001
		Site x survey	3	47.966	2.053	0.147
	6 rings	Site	1	14.577	2.243	0.156
		Survey	3	57.920	8.912	0.001
		Site x survey	3	62.627	9.636	0.001
	∑PAHs	Site	1	39673.736	9.818	0.006
		Survey	3	131679.780	32.587	<0.001
		Site x survey	3	16972.471	4.200	0.023

df - degrees of freedom; F – F statistic; *p* – *p* value (probability level for significant effects: $p < 0.05$).

4.3.2 Total ash PAHs contents

Figure 17 summarize the total contents of the 15 USEPA prioritized PAHs of the collected ash samples. The total PAHs contents of the ashes were higher than those of the underlying topsoil at all four study sites and at all sampling occasions. The differences in average contents ranged from a factor 2.4 to a factor 7.8, with a tendency to be smaller immediately after the fire (2.4–3.1) than afterwards (3.1 -7.8). This tendency was due to smaller relative decreases with time-since-fire in the case of the ash samples than of the soil samples, at both the eucalypt and the pine site in Ermida, and most notably between months 4 and 8 after the fire (eucalypt: -17 vs. -55 %; pine: -15 vs. -52 %). Overall, the ashes from both study sites in Ermida revealed a more gradual decrease in total PAHs content with time-since-fire than the respective soils.

Similar to the soil samples, the ash samples from the eucalypt plantation revealed a noticeable increase in total PAHs content between months 8 and 15, while the ash samples from the pine plantation did not. Nonetheless, this increase in total ash PAHs content at the eucalypt site was clearly less pronounced than that in total soil PAHs content (+12 vs. +60 %). In terms of statistically significant differences, however, the temporal patterns in total ash PAHs contents matched those in total soil PAHs contents, with a significant decrease only observed during the first 4 months after the fire at both the eucalypt and the pine plantation in Ermida.

The total PAHs contents of the ash samples were consistently higher at the pine plantation than at the eucalypt plantation in Ermida, as was the case for the soil samples. However, the differences between the pine and eucalypt ash samples were markedly smaller than those between the soil samples, except immediately after the fire (+52 % vs. +3 to +18 % vs. +52 to +64 %). Equally in agreement with the results for the soils, the ashes of the pine plantation in S. Pedro do Sul had a significantly higher total PAHs content than the ashes of S. Pedro do Sul eucalypt plantation. This difference amounted to 36 %, just above the 30 % for the soil samples. A final concordance with the findings for the soil samples was that the ashes of the S. Pedro do Sul sites had clearly lower total PAHs contents than the ashes of the Ermida sites (eucalypt: -31 %; pine: -38 %) but neither of these differences was statistically significant.

4.3.3 Soil PAHs profiles

The PAHs distribution patterns (3 to 6 rings) in soils of Ermida and S. Pedro do Sul were shown in Figure 18 as well as in Table 12. The contributions of the PAHs with 3- and 4-rings (66-91%) were much higher than those of PAHs with 5- and 6-rings (9-34 %) throughout unburnt and burnt soils for all sampling periods. The 4-rings PAHs (43 %) clearly dominated the unburnt eucalypt soil, followed by the 3-rings PAHs (27 %), whilst the 5- and 6-rings PAHs presented lower contributions of 17 % and 13 %, respectively. However, a shift in the dominance was observed for the burnt eucalypt soil: the 3-rings PAHs compounds was the dominant group, accounting for 69 % of the total PAHs, followed by the 4-rings (22 %), whilst the 5- and 6-rings PAHs were under represented (7 % and 2 %, respectively). Worth mention that significant differences (p<0.05) between unburnt and burnt eucalypt soils collected IAF were only found for the 3- and 4-rings groups. No temporal changes were found for the distribution patterns of the unburnt eucalypt soils. A different temporal trend was observed for the burnt eucalypt soil in Ermida. The contribution of the 3-rings PAHs (33 %) to the total PAHs decreased

considerably 4MAF, whereas the contribution of PAHs with 4- to 6-rings increased to 33%, 21 % and 13 %, respectively. This trend remained unchanged 8 and 15MAF. In general, significant statistically differences were found between the first and second sampling occasion, for all the groups.

The burnt pine soil in Ermida exhibited a similar composition and temporal trend as the burnt eucalypt soil. The 3- and 4-rings PAHs were the principal groups in the four sampling occasions. The 3-rings PAHs revealed a striking shift in their contribution to the total PAHs 4MAF, from 60 % to 47 %, whilst the 4- to 6 rings showed an opposite trend, from 29 % to 33 %, 8 % to 14 % and 3 % to 13 %, respectively. This initial difference in the temporal profile was, in general, the only one that was statistically significant, in agreement with the statistical test outcomes for the burnt eucalypt soil. On the others two sampling occasions (8 and 15MAF) the contributions of the different groups to the total PAHs levels were basically in the same extend as in 4MAF. The burnt eucalypt and pine soils of S. Pedro do Sul, showed a similar composition profile as the burnt eucalypt and pine soils of Ermida - it was dominated by the 3-ring PAHs (eucalypt: 70 %; pine: 56 %) followed by the 4-ring PAHs (eucalypt: 22 %; pine: 28 %), whilst the 5- and 6-ring were less representative (eucalypt and pine:< 9 %).

In terms of the individual PAHs, four compounds, fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT) and pyrene (PYR), dominated the different samples collected in Ermida (Table 1). Three of them - PHE (18-24 %), FLT (19-23 %) and PYR (14-18 %) – were the principal components in the unburnt eucalypt soils at the four sampling occasions, whereas the PHE (44 %) and FLU (14 %) were dominant IAF for the burnt eucalypt soil. Fluoranthene and PYR accounted each with 9 % and 11 %, respectively, to the total PAHs. In general, 4, 8 and 15MAF, PHE and FLU in burnt soils showed decreased contribution, accounting for 21-27 % and 7-9 %, of the total PAHs, respectively, whereas the FLT contributed 13-18 % to the total PAHs, basically to the same extend as PYR (12-15 %).

The individual contributions to the total PAHs of the burnt pine soil in Ermida were similar to those of the burnt eucalypt soil. Immediately after the fire, PHE accounted for 37 %, whereas FLU, FLT and PYR, accounted each for 11 %. Four, 8 and 15MAF, a decreased in the contribution of FLU (5-7 %) and PHE (24-35 %) was observed. Contrary, FLT (14-18 %) and PYR (13-14 %) increased in their contributions. Worth mentioning the striking shift in the contribution of IND for burnt eucalypt and pine soils, with time since fire, from 0.1 % and 0.3 % (IAF) to 4 % and 10 % (4MAF), respectively. Also in the S. Pedro do Sul area, the burnt eucalypt and pine soils profiles were dominated by PHE (eucalypt: 47 %; pine: 28 %), FLU (eucalypt: 12 %; pine: 14 %), PYR

(eucalypt: 12 %; pine: 13 %) and FLT (eucalypt: 6 %; pine: 11 %). Important to stress the contribution of the 6-ring PAH, IND, 6 % of the total PAH in burnt pine soil of S. Pedro do Sul.

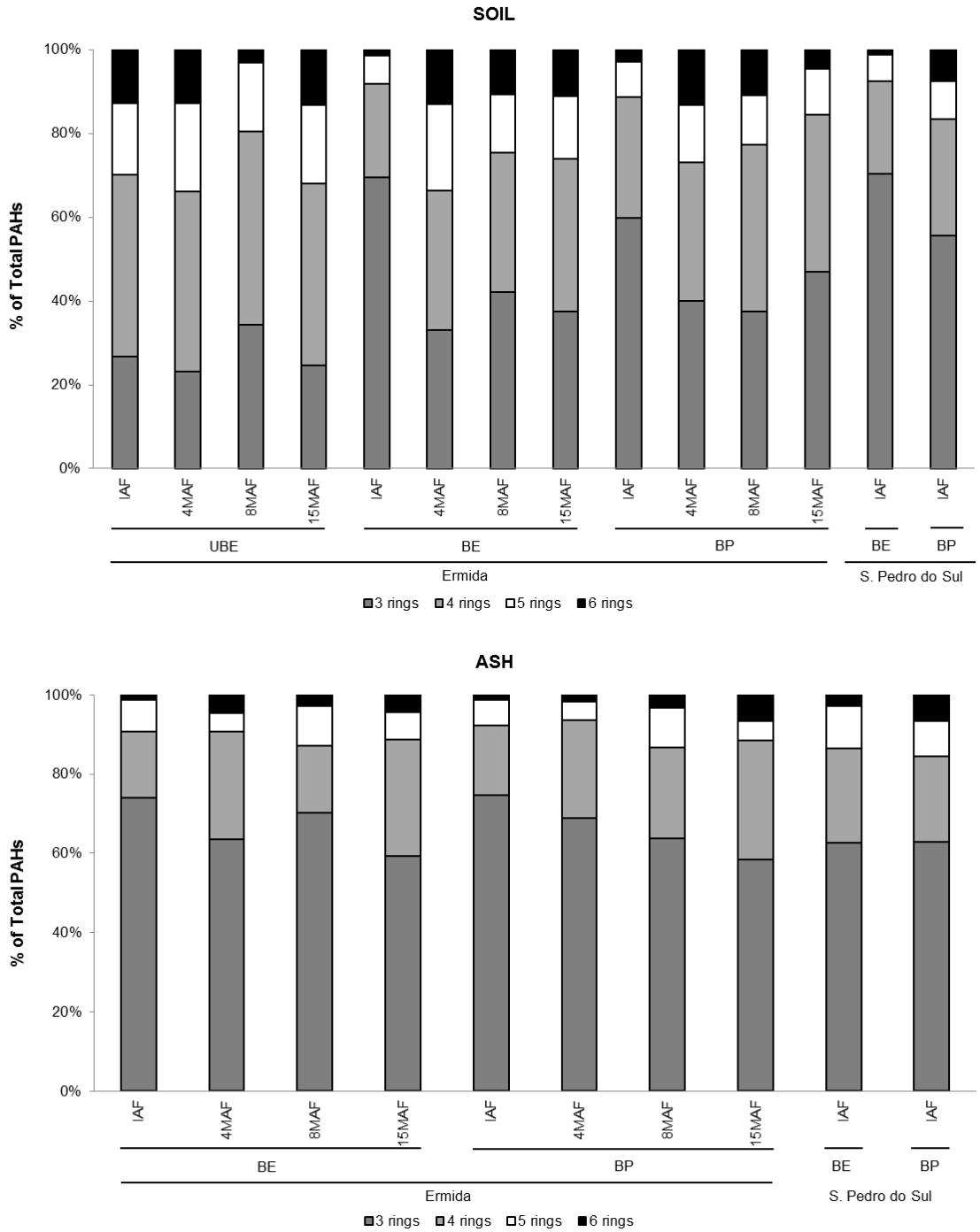

Figure 18 - Composition profiles of mean PAH contents in soil and ash samples collected in unburnt (UBE) and burnt (BE) eucalypt plantations and burnt pine plantations (BP) in Ermida and S. Pedro do Sul immediately and 4, 8, 15 months after fire (IAF, 4MAF, 8MAF and 15MAF, respectively).

Table 12 - **–** Mean concentration and associated standard deviation of individual PAHs (ng g^{-1}) and total content by number of aromatic rings in soils collected immediately after fire (IAF), 4 months after the fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF) at Ermida and S. Pedro do Sul. *Asterisks* indicate significantly differences for PAHs levels between long-unburnt (UBE) and burnt eucalypt sites at Ermida (BE). Different letters and symbols denote statistically significant differences (p<0.05) between sites (for the same period sampling) or between sampling periods (for each site), respectively.

PAHs	rings	ERMIDA												S. PEDRO DO SUL	
		UBE				BE				BP				IAF	
		IAF	4MAF	8MAF	15MAF	IAF	4MAF	8MAF	15MAF	IAF	4MAF	8MAF	15MAF	BE	BP
ACY	3	0.47±0.02	1.1±0.18	0.70±0.02	0.65±0.02	6.9±1.72	1.6±0.05	1.0±0.10	1.0±0.17	14.1±5.51	2.6±0.81	1.5±0.01	1.6±0.52	5.8±0.68	14.2±2.88
ACE	3	<0.50	<0.50	<0.50	<0.50	0.91±0.42	<0.50	<0.50	<0.50	1.7±0.74	<0.50	<0.50	0.61±0.10	0.66±0.20	1.8±0.53
FLU	3	<0.50	0.76±0.01	1.1±0.10	0.96±0.19	20.4±3.43	5.0±1.69	2.9±1.28	3.4±1.54	27.0±6.25	7.1±2.06	2.6±0.55	4.8±1.51	16.1±2.07	24.7±5.75
PHE	3	8.4±0.93	9.3±2.40	11.0±3.52	8.8±1.99	65.7±10.22	14.5±4.04	8.4±2.43	13.3±4.47	90.3±24,63	29.4±4.09	11.9±3.22	28.1±8.28	61.8±7.85	47.3±2.91
ANT	3	0.91±0.07	1.1±0.22	0.73±0.26	<0.30	8.8±0.69	2.2±0.62	1.0±0.33	1.3±0.33	12.1±4.10	3.6±1.52	2.3±0.35	2.6±1.21	8.4±1.40	7.3±0.63
FLT	4	8.0±1.52	9.8±2.41	8.7±2.71	8.5±1.50	13.5±2.95	10.3±2.84	4.6±0.33	7.8±2.70	26.4±5.82	15.0±4.78	8.8±1.56	12.5±4.03	8.6±1.23	19.2±3.45
PYR	4	5.5±0.21	8.6±1.89	6.9±3.11	5.7±0.66	13.3±3.04	9.6±1.84	3.7±0.40	6.1±2.21	27.2±7.44	13.5±4.11	6.9±2.49	10.7±4.71	15.2±4.79	21.6±6.44
BaA	4	0.79±0.29	1.5±1.04	0.87±0.31	1.5±0.63	4.6±1.22	1.9±0.71	0.88±0.20	1.9±1.40	9.6±3.21	3.0±0.85	1.6±0.68	2.8±0.87	4.6±1.06	4.1±0.48
CHR	4	1.5±1.03	2.7±2.57	1.7±0.26	2.5±1.01	1.7±0.94	1.7±0.32	1.4±0.12	2.7±1.04	6.8±2.40	3.7±0.37	2.2±0.87	4.2±0.78	0.72±0.13	2.6±0.50
BbF	5	1.8±0.77	6.6±1.44	3.8±1.12	4.5±0.71	3.4±1.18	6.3±1.16	2.0±0.43	3.6±1.78	9.7±1.70	6.2±1.68	2.7±0.53	4.2±0.69	1.9±0.16	4.8±0.41
BkF	5	0.56±0.04	1.5±0.13	0.70±0.02	2.0±0.72	0.96±0.15	1.6±0.28	0.94±0.15	1.8±0.78	1.3±0.61	1.5±0.48	1.2±0.30	1.9±0.51	<0.50	0.65±0.26
BaP	5	3.8±0.42	2.9±0.86	1.9±0.36	1.4±0.24	5.4±0.64	6.7±1.30	1.4±0.34	2.2±0.95	8.9±2.24	6.9±1.61	1.9±0.27	2.6±0.59	6.7±1.22	7.1±1.11
DBahA	5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.65±0.06	<0.50	<0.50	<0.50	<0.50	2.9±0.42
IND	6	2.1±1.13	2.4±0.92	0.57±0.04	2.5±0.77	<0.50	2.6±0.49	1.7±0.52	2.8±1.16	0.63±0.02	10.8±2.53	2.7±0.75	1.6±0.33	<0.50	10.1±2.25
BghiP	6	2.6±0.03	4.4±1.19	0.68±0.09	3.0±0.14	2.1±0.60	6.6±1.25	1.7±0.41	2.9±1.32	6.2±0.01	3.4±0.59	2.6±0.85	2.2±0.45	1.5±0.60	2.9±0.23
3 rings		9.8 ∇	12.3 ∇	13.5 ∇	10.4 ∇	103 ∗a∇	23.3 ∗a◊	13.3 a◊	19.0 ∗a◊	145 b∇	42.7 b◊	18.3 a□	37.7 b◊	92.8 a	95.3 a
4 rings		15.8 ∇	22.6 ∇	18.2 ∇	18.2 ∇	33.2 ∗a∇	23.5 a◊	10.6 a◊	18.5 a◊	70.0 b∇	35.2 b◊	19.5 a◊	30.2 a◊	29.1 a	47.5 a
5 rings		6.2 ∇	11.0 ◊	6.4 ∇	7.9 ∇◊	9.8 a∇◊	14.6 a∇	4.3 a◊	7.6 a◊	20.5 b∇	14.6 b◊	5.8 a□	8.7 a□	8.6 a	15.5 b
6 rings		4.7 ∇	6.8 ∇	1.3 ◊	5.5 ∇	2.1 a∇	9.2 a◊	3.4 a∇□	5.7 a□	6.8 b∇	14.2 b◊	5.3 a∇	3.8 a∇	1.5 a	13.0 b

Compounds abreviations: acenaphthylene (ACY), acenaphtene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT); fluoranthene (FLT), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR); benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(ah)anthracene (DBahA), indeno(1,2,3-cd)pyrene (IND) and benzo(g,h,i)perylene (BghiP).

4.3.4 Ash PAHs profiles

The composition patterns of PAHs by ring size in ashes from Ermida and S. Pedro do Sul were presented in Figure 18 as well as in Table 13. Similar to the burnt eucalypt and pine soil pattern, the 3-rings PAHs exhibited predominance (approx. 75 %) over 4-rings (approx. 17 %), 5-rings (approx. 7 %) and 6-rings (approx. 1 %) in the ash samples collected IAF. Although the 3-rings dominated the contributions to the total PAHs over the other groups at the four sampling occasions, those predominance revealed a decreased with time since fire to approximately 60 %. The profile of the 4- and 6-rings PAHs increased with time since fire to approximately 30 % and 5 %, respectively. In general, the contribution of the 5-rings PAHs remained unchanged along the sampling period. Equally in agreement with the results for the Ermida, the ashes from eucalypt and pine plantations of S. Pedro do Sul (Figure 18) were dominated by the 3-rings PAHs (65 %), followed by the 4-rings (approx. 22 %), whereas the 5- and 6-rings were under-represented (approx. 8 % and 5 %, respectively).

As to individual PAHs, the relative composition of the PAHs in eucalypt and pine ashes collected IAF in Ermida was dominated by two compounds: PHE (33 %) and FLU (approx. 26 %) followed by the FLT and PYR (8-12 %, respectively).The remain individual PAHs accounted for less than 7% each. Although, similar relative PAHs compositions were found in the four sampling events, eucalypt and pines ashes revealed an increased contribution of PHE to approximately 40 % and a decreased contribution of FLU to approximately 10 % with time since fire. A slightly increased contribution of FLT and PYR to approximately 12% with time since fire was also observed for eucalypt and pine ashes from Ermida. In agreement with the findings for the ashes samples from Ermida was that the eucalypt and pine ashes of the S. Pedro do Sul sites was clearly dominated by PHE (eucalypt: 45 %; pine: 34 %) followed by FLU (eucalypt: 10 %; pine: 14 %), FLT (eucalypt: 11 %; pine: 9 %), PYR (eucalypt and pine: 11 %) and FLT (eucalypt: 11 %; pine: 9 %).

Table 13 - Mean concentration and associated standard deviation of individual PAHs (ng g^{-1}) and total content by number of aromatic rings in ashes collected immediately after the fire (IAF), 4 months after the fire (4MAF), 8 months after the fire (8MAF) and 15 months after the fire (15MAF) at Ermida and S. Pedro do Sul. Different letters and symbols denote statistically significant differences (p<0.05) between sites (for the same period sampling) or between sampling periods (for each site), respectively.

PAHs	rings	ERMIDA								S. PEDRO DO SUL	
		BE				BP				IAF	
		IAF	4MAF	8MAF	15MAF	IAF	4MAF	8MAF	15MAF	BE	BP
ACY	3	35.2±6.72	12.1±2.50	8.8±2.30	11.2±4.36	45.2±5.47	17.9±3.52	14.8±1.90	9.2±4.22	11.2±2.88	36.3±14.57
ACE	3	10.6±1.71	4.4±1.02	2.4±0.18	6.4±1.39	8.6±0.86	15.3±5.36	3.0±0.78	10.8±1.19	1.8±0.53	4.9±1.24
FLU	3	118±9.88	38.8±10.51	24.1±2.97	29.4±7.29	202±11.20	65.4±27.0	39.9±11.76	23.9±4.89	21.1±4.93	61.3±13.86
PHE	3	151±18.10	118±21.30	122±6.06	107±10.68	229±18.50	123±46.12	107±13.37	113±36.93	142±15.48	146±13.79
ANT	3	25.1±6.25	9.9±2.49	14.9±1.33	9.4±2.11	36.1±3.59	11.6±5.45	14.6±1.28	9.3±3.93	20.8±.4.47	21.4±6.07
FLT	4	35.2±5.45	33.2±4.96	18.4±2.89	32.2±6.01	53.3±5.38	36.6±17.86	24.4±4.32	35.8±10.75	33.7±1.21	37.1±6.33
PYR	4	36.1±6.13	25.8±3.56	19.8±4.86	27.8±5.77	55.6±4.97	30.0±14.22	26.3±4.87	30.5±7.37	35.7±0.70	48.4±9.10
BaA	4	4.7±0.72	7.1±2.17	2.9±0.44	8.9±0.98	10.2±0.96	7.7±2.18	5.5±1.26	6.6±0.95	2.9±0.76	5.2±0.68
CHR	4	1.3±0.41	11.7±1.39	<0.60	11.8±1.79	4.2±0.81	10.1±3.80	8.3±0.24	12.5±2.48	2.7±0.17	1.6±0.28
BbF	5	23.8±4.93	7.5±1.59	15.3±2.51	9.5±1.29	29.9±4.50	8.1±3.38	13.7±0.67	9.5±2.59	22.0±2.69	12.0±2.33
BkF	5	3.2±0.50	3.1±1.37	5.3±1.55	4.1±1.03	2.9±0.29	3.8±1.48	3.2±0.63	4.2±1.25	4.5±2.48	1.9±0.89
BaP	5	9.8±1.14	3.2±0.99	4.3±1.26	5.3±1.78	10.3±1.90	4.3±0.83	8.2±1.95	<0.60	5.8±0.17	19.6±5.55
DBahA	5	<0.50	<0.50	<0.50	<0.50	2.5±0.47	<0.50	2.8±0.81	<0.50	1.0±0.29	4.7±0.66
IND	6	3.6±0.71	13.1±3.36	3.0±0.27	6.8±0.60	3.2±0.53	5.4±1.44	4.3±1.62	18.9±5.26	4.1±0.40	23.1±5.36
BghiP	6	1.8±0.04	<0.70	3.7±0.17	5.4±0.20	5.2±0.80	<0.70	5.1±0.61	<0.70	5.0±0.29	5.1±0.54
3 rings		340 a∇	183 a◊	172 a◊	163 a◊	521 b∇	234 a◊	179 a◊	166 a◊	197 a	270 b
4 rings		77.2 a∇	77.8 a∇	41.1 a◊	80.7 a∇	121 b∇	84.4 a◊	64.5 a◊	85.4 a◊	75.0 a	92.3 a
5 rings		36.8 a∇	13.8 a◊	24.9 a◊	18.9 a◊	43.8 a∇	16.2 a◊□	27.9 a□	13.7 a◊	33.3 a	38.2 a
6 rings		3.6 a∇	13.1 a◊	6.7 a∇	12.2 a◊	8.4 a∇	5.4 b∇	9.4 a∇	18.9 a◊	9.1 a	28.2 b

Compounds abreviations: acenaphthylene (ACY), acenaphtene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT); fluoranthene (FLT), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR); benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(ah)anthracene (DBahA), indeno(1,2,3-cd)pyrene (IND) and benzo(g,h,i)perylene (BghiP).

4.3.4 The isomer ratios of PAHs

The PAHs isomer ratios of FLT/(FLT+PYR) and BaA/(BaA+CHR) were calculated to identify the possible sources of PAHs in soils (Figure 19). The BaA/(BaA+CHR) ratios reported in this study ranged between 0.34 and 0.38 in unburnt soils and between 0.39 and 0.74 in burnt soils. The ratios of FLT/(FLT+PYR) ranged between 0.53 and 0.60 in the unburnt and between 0.49 and 0.56 in the burnt soils. As it can be seen in Figure 5, these two diagnostic ratios of PAHs were generally positioned in the upper right corner, suggesting a greater influence from combustion of grass, wood and coal combustion, although the ratio FLT/(FLT+PYR) did not confirm a clear trend for all samples (e.g., the burnt soils from S. Pedro do Sul were more indicative of petroleum combustion).

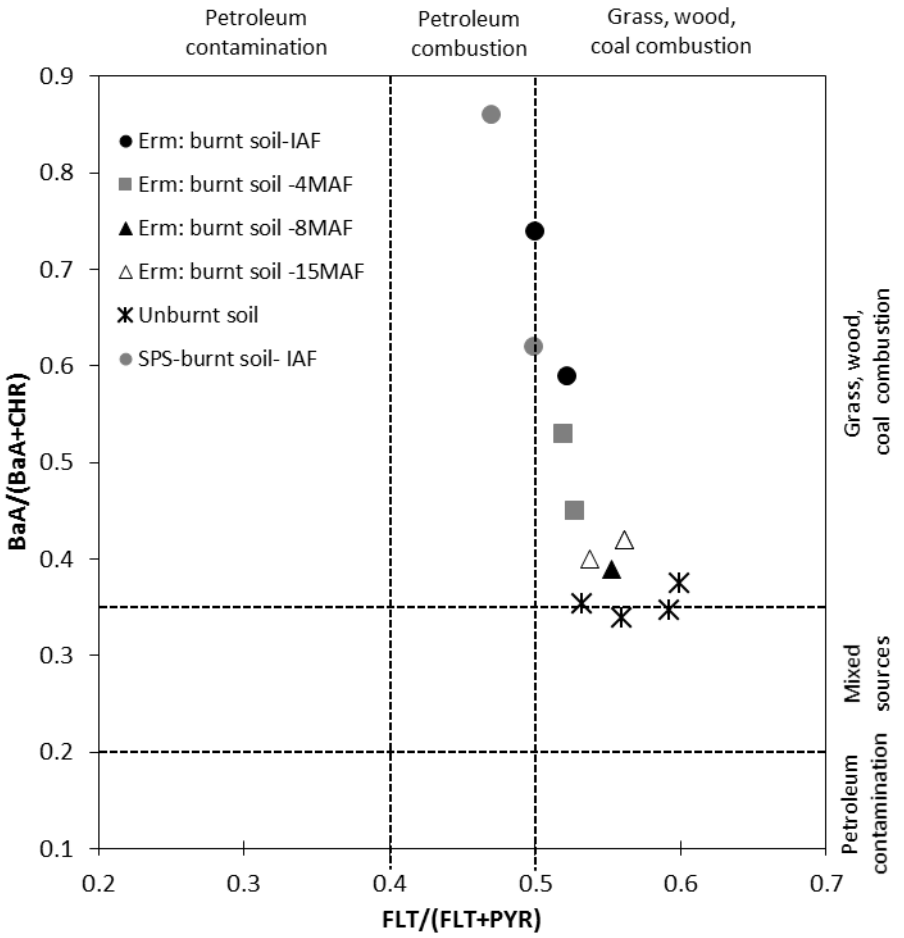

Figure 19 – Mean FLT/(FLT+PYR) vs. BaA/(BaA+CHR) isomeric ratios in soil samples collected in unburnt and burnt plantations in Ermida (Erm) and S. Pedro do Sul (SPS). The limits of the isomeric ratios were adapted from Yunker et al. (2002).

4.3.4 Soil and ash organic matter content

The organic matter (OM) contents of the collected soil and ash samples as well as the associated statistically significant differences were resumed in Figure 17. The results for the Ermida eucalypt plantations suggested a clear and persistent effect of wildfire occurrence on soil organic matter (SOM) content. At all four sampling occasions, the SOM content was significantly higher at the burnt than unburnt eucalypt plantation (19-24 % vs. 16-17 %). However, the SOM content at the burnt eucalypt plantation revealed a tendency to decrease with time-since-fire, whereas SOM content at the unburnt eucalypt plantation did not. This decrease with time-since-fire seemed rather gradual, even though SOM content was significantly lower at the last two sampling occasions than at the first two. Also the actual SOM contents of the burnt pine plantation in Ermida were similar to those at the burnt eucalypt plantations in Ermida, not differing significantly at any of the four sampling occasions. In contrast, the SOM contents of the two S. Pedro do Sul plantations immediately after the fire were markedly lower than those of the two burnt plantations in Ermida (10-12 % vs. 22-240 %). The absence of significant differences between types of vegetation (eucalypt vs. pine) for SOM was also observed in S. Pedro do Sul. The relationship between PAHs concentrations and SOM were assessed using the Spearman coefficients correlations. The results of these correlation analyses indicate that the total PAHs concentrations were moderately and highly correlated with SOM in soils of Ermida ($r = 0.593$, $p < 0.001$) and of S. Pedro do Sul ($r = 0.938$, $p < 0.001$), respectively.

Organic matter content in ashes (AOM) exceeded the values measured in soil for both areas, Ermida and S. Pedro do Sul. Likewise the SOM content, the AOM content showed a decrease with time-since-fire at both burnt plantations in Ermida. However, this decrease in AOM content was more abrupt and shorter-lived than that in SOM content, especially at the burnt pine site where it almost halved from 37 % to 19 % during the first four months after the fire and then remained basically unaltered over the rest of the study period. At all four sampling occasions, the AOM contents tended to be lower for the burnt pine plantation than for the burnt eucalypt plantation in Ermida, but none of these differences was statistically significant. In the S. Pedro do Sul area, however, the AOM content of the burnt pine plantation was significantly higher than that of the burnt eucalypt plantation. Nonetheless, the AOM contents of the two S. Pedro do Sul sites immediately after the fire were significantly lower than those of the burnt Ermida sites, as was the case for the SOM contents. As in the case of soils, PAHs levels in ashes from

Ermida ($r = 0.721$, $p < 0.001$) and S. Pedro do Sul ($r = 0.900$, $p < 0.001$) were strongly correlated to AOM.

4.4 Discussion

4.4.1 The levels of PAHs in soil and ash

In general terms, the total PAH contents of the unburnt eucalypt soil in Ermida were low and typical for uncontaminated soils with reduced influence of anthropogenic PAH sources (Maliszewska-Kordybach, 1996). The total PAH contents in the unburnt eucalypt soil in Ermida (34.5-52.7 ng g-1) were comparable to those reported by various studies of unburnt soils in different forest types around the world. They included a forest in South Korea (45.5 ng g-1; Kim et al., 2003; 2011), a forest in the mountains of Western Canada (30.0 ng g-1; Choi et al., 2009), Quercus and Pinus forest in France (∑14 EPA-PAH content of 40 ng g-1; Vergnoux et al., 2011) and a forest in a Nature Reserve of China (22 ng g-1; Xiao et al., 2014). Weiss et al. (2000) and Nam et al. (2008) however, observed higher total PAH contents in unburnt Norway spruce and coniferous and deciduous soils, amounting to 210 ng g-1 and to values up to 580 ng g-1, respectively. The differences among studies may be a function of the forest type differences, the proximity to PAH sources resulting in greater atmospheric emissions and subsequent deposition and retention in soil and loss processes (e.g. leaching, biodegradation and volatilization). These processes are all influenced by compound and soil/environmental properties (Nam et al., 2008).

The total soil PAH contents observed in this study immediately after the fire (148 and 242 ng g-1 in eucalypt and pine stands of Ermida, respectively, and 132 and 171 ng g-1 in eucalypt and pine stands of S. Pedro do Sul, respectively) were similar to those of a mangrove forest in Nigeria two months after the fire (60.5-181 ng g-1, Sojinu et al., 2011) and in a forest in South Korea one month after the fire (112 ng g-1; Kim et al., 2011). Kim et al. (2003; 2011),however, also reported much higher values for forest soil in the eastern coastal region of South Korea one month after the fire (502 - 1302 ng g-1). The wildfire seemed to have had a pronounced direct effect on the levels of PAHs in soils, pointing to an extremely high incorporation of PAHs compounds in burnt eucalypt soils IAF (approximately 4.3 times) in comparison to the unburnt eucalypt soil of Ermida. This hypothesis is reinforced by the fact that the studied areas were situated far from cities or industrial areas, being as much as possible preserved from anthropogenic impacts. This way, we could assumed minimum risk of interference from other inputs

than the wildfire. The increased levels of PAHs observed in burnt soils of Ermida IAF were consistent with the formation of PAH by combustion of biomass (wood, other vegetation and the litter layer) during wildfires that has been previously reported by several authors (Jenkins et al., 1996; Conde et al., 2005; Olsson and Kjallstrand, 2006). It is worth mentioning that the formation of PAHs within soil during fires (for example, by the combustion of soil organic matter) must also be considered. The PAH formed during combustion remain in the gas phase at temperatures above 150°C [during the wildfire in the Ermida area, the burnt soil reached temperatures higher than 150°C (Campos et al., 2015)], but rapidly condense onto fly-ash particles at lower temperatures (Wilcke, 2000). This way, PAHs are probably adsorbed by ash particles, then finally deposited onto the surface soil, suggesting that the concentration of PAHs in soil were strongly influenced by the ashes (ash-derived PAHs) resulting from the combustion of wood and litter layer (Kim et al., 2003). This hypothesis is reinforced by the fact that the levels of PAHs in ashes in our study were much higher than those in the corresponding soil samples, which was also reported by Kim et al. (2003; 2011). Indeed, the present study revealed that ash from burnt vegetal biomass and litter layer was comprised of elevated levels of PAHs. The PAHs levels in ash samples collected IAF in Ermida (458 and 695 ng g-1) and S. Pedro do Sul (315 and 429 ng g-1) are similar (355 and 589 ng g-1) to those reported by Kim et al (2011) and much higher (∑12 PAHs, 1.3, 14 and 19 ng g-1) compared to the one´s found by Olivella et al. (2006). However, the levels are much lower (∑7 PAHs 690, 1354, 1427 and 1612 ng g-1) than those of ashes produced in the laboratory (Rey-Salgueiro et al., 2004).

The PAHs concentrations in pine burnt soils were higher than the values measured in the eucalypt one´s, for both surveyed areas, Ermida and S. Pedro do Sul. These differences can be related with the higher levels observed in the ashes from the pine sites compared to the eucalypt ashes. The differences observed between pine and eucalypt soils and ashes suggest that pine reveals a stronger entrapment affinity towards PAHs than eucalypt. Note that the difference in OM content between pine and eucalypt soils and ashes were insufficient to account for the difference in PAHs levels. Rey-Salgueiro et al. (2004) also found higher levels in pine ashes than in eucalypt ashes (∑7PAHs: 1427 ng g-1 versus 690 ng g-1) and the same was true for Olivella et al. (2006) that observed differences in PAHs levels in ashes from different vegetations (oak versus pine). Furthermore, Ratola et al. (2010) observed different PAHs levels between needles of Pinus pinea and Pinus pinaster. These studies corroborates that different plant species can favour the entrapment of PAHs levels. Nevertheless, further studies

should confirm whether pines, in comparison to eucalypts, accumulate more PAHs levels, creating conditions to their retention in ashes.

The relationship between PAHs levels and fire severity was not straightforward, since significant differences ($p<0.05$) were only detected for ashes, although our results showed a tendency to have higher levels of PAHs in soils and ashes from Ermida (moderate severity) compared to S. Pedro (high severity). However, Kim et al. (2011) found higher levels of PAHs in burnt soils of severe forest fires (547, 660 and 1302 ng g-1) than in burnt soils of less severe fires (502 and 112 ng g-1). Hence, differences in fire severities may accounted for the different extents of the formation of PAHs between Ermida and S. Pedro do Sul.

The effect of the wildfire on burnt soils was short-lived as the total PAH contents in burnt soils decreased to pre-fire levels within 8 months after the fire. Similar behavior was also reported by Kim et al. (2003, 2011) with an initial increased of PAHs levels in burnt soils after the forest fire followed by a rapid decrease (-85 %) nine months after the fire, and by Garcia – Falcón et al. (2006) in a Atlantic woodland where the PAHs content in burnt soils collected ten months after the fire, fell to about 57% of their peak level (ten days after the fire). Worth mentioning that Vergnoux et al. (2011) also demonstrated the contribution of forest fires to the content of PAHs in burnt soils as well as the decreased of their total levels with time. Such decreased in PAHs levels of burnt soils and ashes with time (mainly 4MAF) was probably due to selective leaching into lower soils layers and washout of PAHs polluted soil and ash (erosion) associated to particles by heavy rainfall in winter, as also suggested by Kim et al. (2003; 2011), Garcia-Falcón et al. (2006) and Vergnoux et al. (2011), even though PAHs are known to have a low overall mobility in the soil columns (Jones et al., 1989; Krauss et al., 2000; Vergnoux et al., 2011). However, degradation of PAHs (mainly biological degradation) could also have played a relevant role, in spite PAHs are typically considered to be persistent in the environment and have a low mobility in soils (Jones et al., 1989; Krauss et al., 2000; Vergnoux et al., 2011). A point of agreement that could also justify why PAHs content in burnt soils decrease with the time elapsed since the fire was the temporal evolution in the PAHs profiles (point 4.2), where we observed a preferential disappearance of the less persistent PAHs (3-ring) which were, in general, generated by the wildfire.

Several studies have clearly pointed out that heavy rainfall and the subsequent surface runoff play an important role in soil erosion and therefore on the transport of particles to downstream aquatic systems (Robichaud et al., 2009; Malvar et al., 2011; Prats et al., 2014). These events are considered important inputs of contaminants from diffuse sources to water systems in burnt areas (Bundt et al., 2001; Rey-Salgueiro et al.,

2004; Olivella et al., 2006; Vila-Escalé et al., 2007; Campos et al., 2012; Silva et al., 2014). Particularly, the first heavy rains after the wildfire caused an accentuated surface washing over the burnt areas of Ermida, which was attenuated over time (Martins et al., 2013; Prats et al., 2014). These phenomena of transport towards neighbourhood groundwater systems and water flows or rivers in areas affected by wildfires was also emphasised by previous studies that showed an increase of PAHs levels in groundwater (Mansilha et al., 2015) and water and sediments (Gabos et el., 2001; Olivella et al., 2006; Vila-Escalé et al., 2007; Schäfer et al., 2010; Smith et al., 2011; Campos et al., 2012) after a wildfire and heavy rainfall.

The slight increase in the levels of PAHs in burnt soil observed between 8 (summer) and 15MAF (winter), although not statistically significant ($p>0.5$) could be attributed to seasonal variations in the levels of PAHs in soils. This could involve increased deposition of atmospheric PAHs during cold weather conditions, resulting from increased domestic space heating, garden residues burning, reduced thermal and photodecomposition and reduced atmospheric mixing (Wilcke, 2000; Kim et al., 2011). However, in the unburnt soil this slight increase was not noted. Kim et al. (2011) also observed an increased in PAHs levels of 3.0 and 1.3 times in burnt soils between five and nine months after the forest fire.

Overall, the temporal behaviour of burnt soils and ashes were very similar, suggesting that the main source influencing the levels of PAHs in the burnt soil was likely to be the deposition of the ash.

4.4.2 Composition of PAHs in soil and ash

The composition pattern of PAHs by ring size and in terms of individual PAHs identified in this study were, in general, similar to those established for burnt forest soils by Kim et al. (2011) and Vergnoux et al. (2011), being dominated by 3- and 4-ring PAHs and showing significant differences from the unburnt in samples collected IAF. The remarkably higher levels reached by the 3-rings PAHs (PHE and FLU) and 4-rings PAHs (FLT and PYR) in the burnt soil was in good harmony with the fact that they are the most produced aromatic compounds during wildfires (Yuan et al., 2008). Furthermore, the levels of the 5- to 6-ring were not significantly different between unburnt and burnt soils, and in some cases, they are higher in the unburnt soil, indicating that probably the forest fire did not generate the heaviest PAHs (Vergnoux et al., 2011). Worth mentioning the exception in behaviour of the BaP (5-ring PAHs compound), showing amounts increased

by a factor about 3 in burnt soil than in unburnt soil in the first two sampling campaigns, as it is classified as one of the most potent carcinogenic and mutagenic PAHs. As well known, 3- to 4-ring PAHs are chiefly generated by low- or moderate temperature combustion process, such as biomass combustion and domestic coal burning, while 5- to 6-ring PAHs are mainly generated by high-temperatures combustion process, which are associated with traffic (such as vehicular exhausts) and industrial coal combustion (Orecchio et al., 2008; Singh et al., 2008). Additionally, PHE, FLT and PRY, beside the NAP (that wasn't analysed in our study) are considered the dominant PAHs in airborne particulate matter derived from wood combustion rather than BghiP, DBahA and IND (Freeman and Cattell, 1990; Xu et al., 2006). PYR is also considered, along with PHE and FLT, as one of the major PAHs products resulting from the burning of gymnosperms and angiosperms biomass (Simonelt, 2002). Furthermore, the most volatile PAHs, such as NAP, FLU, PHE and ANT, as well as the FLT (less volatile than the previous mentioned) appear to be the major PAHs produced by pinewood and needles combustion (Schauer et al., 2001; Conde et al., 2005). Those facts in addition to our results suggest that the forest fire was the major factor affecting the PAHs profiles in burnt soil. The high proportion of the 3-ring PAHs (PHE and FLU) compounds followed by the 4-ring (FLT and PYR) in burnt soil were also shared by the ash samples, whereas in unburnt soil it was the opposite, which might indicate the common source and confirm the previous observations. Similar PAHs profiles in terms of rings and individually PAHs compounds in ashes from a forest fires were also reported in Korea (Kim et al., 2011).

The temporal decreased (from 69% to 33%) in the contribution of the 3-ring PAHs compounds (ACY, ACE, FLU, PHE and ANT) 4MAF whereas the contribution of the PAHs with 4 to 6-rings increased (from 22% to 33%, 7% to 21% and 2% to 13%, respectively) and with the increase of time elapsed since fire, caused the PAHs profile in burnt soils became more closely resemble that of the unburnt soils. The corresponding ashes also followed these temporal patterns of PAHS profiles in burnt soils. This can be explained by the fact that light molecular weight (LMW) PAHs with 3-ring was enhanced by the deposition of ash and then, they were more rapidly removed from the burnt soils because the first rains after fire caused an accentuated washout and leaching of ash that were overlying in the burnt soil, which was attenuated over time. As our results showed, fires generated LMW PAHs, which are more water soluble and volatile, and hence more available than high molecular weight (HMW: 4-to 6-ring) strongly adsorbed onto soil particles. The generally higher availability of LMW PAHS, as compared to HMW PAHs, makes them more susceptible to various biological biodegradation, chemical and photochemical degradation processes and leaching. In regards to HMW PAHs, they are

more recalcitrant when it comes to undergoing degradation processes (Park et al., 1990; Wilcke et al., 2002 Hansen et al., 2004; Vergnoux et al., 2011).

4.4.3 Source apportionment by isomeric ratios of PAHs

The anthropogenic release of PAHs can be attributed to petrogenic (petroleum source) and pyrogenic (biomass and coal combustion) origins. Hence, it is critical to identify the sources and amount of contaminant in soils to adopt appropriate management actions. Several PAHs congener ratios, such as BaA/(BaA+CHR) and FLT/(FLT+PYR) have been widely used to distinguish the possible source categories of environmental PAHs (Yunker et al., 2002; Sprovieri et al., 2007; Jiang et al., 2009; Tay and Biney, 2013). Although these indexes are often used, they have to be considered with caution as they might be influenced by selective degradation of individual PAHs, and the ratios can be changed, mainly during the transport of PAHs in the environment and became remarkably different from those of the source emissions (probably, this is not the case in our study since we expect that emission source- wildfire- was near the study area). Moreover, we must keep in mind that diagnostic ratios provide only qualitative information about the contribution of various sources with regards to PAHs contaminations (Wang et al., 2010).

For nearly all samples collected of Ermida and S. Pedro do Sul, the integrated analysis of the data, BaA/(BaA+CHR) and FLT/(FLT+PYR), suggested that the major source of PAHs in soils and ashes was derived from grass, wood and coal combustion. Moreover, the lack of coal industrial activities in those areas (Ermida and S. Pedro do Sul) would make coal-derived PAHs less important, pointing to the forest fire (grass and wood combustion) as the principal source of PAHs in soils and ashes. Similar features have been observed previously in other soils systems affected by forest fires, which suggested biomass burning as being the major source of PAHs detected in the burnt forest soils and ashes (Olivella et al., 2006; Kim et al., 2011; Sojinu et al., 2011; Vergnoux et al., 2011). In addition, the similarity between the burnt soils and ashes ratios suggest that the wildfire is assumed to be responsible for the measured PAHs. It is interesting to note that BaA/(BaA+CHR) ratio appears to have decrease with time since in the burnt soil [from 0.74 (IAF) to 0.42 (15MAF)], in opposition to the unburnt soil that exhibited relatively uniform ratios (0.34-0.38). Furthermore, the biomass combustion was accentuated for burnt soils because the ratios values of these latter soils were higher

than the unburnt soils and the differences between them became fainter with time since fire.

4.4.4 Association of PAHs with organic matter

The present results suggested that the Ermida wildfire produced a noticeable increase in the SOM content of eucalypt soils and that this direct effect lasted for over one year, in spite of a gradual decrease in SOM content with time-since-fire. Fire-induced increases in SOM content have been reported by various studies across the world and have generally been attributed to new inputs in the form of partly charred litter and leaves, forest necromass and residual ash (Gimeno-Garcia et al., 2000; Pardini et al., 2004; Knicker et al., 2005; Quintana et al., 2007; Boerner et al., 2009). However, decreases in SOM due to fire have been observed as well typically associated to more intense soil heating regimes that result in volatilization or oxidation of SOM (Aref et al., 2011; Granged et al., 2011; Faria et al., 2015; Varela et al., 2015). The markedly lower SOM contents at the S. Pedro do Sul than Ermida sites immediately after the fire were in line with the higher fire severity at the S. Pedro do Sul sites. The same applied for the OM contents of the ash samples collected immediately after the fire in Ermida and S. Pedro do Sul. The extremely high AOM content of the Ermida samples suggested incomplete vegetation combustion and was also in line with their dark color (Goforth et al., 2005).

The gradual decreases of SOM contents in burnt soils of Ermida between sampling periods are usually attributed to slow vegetation recovery and post-fire soil losses as well as the removal of ash from the site by leaching and washout (during rainfall) or erosion (Kim et al., 2003; Campo et al., 2008). This hypothesis is also reinforced by data in control unburnt soil where no statistical differences were found between sampling periods. Furthermore, Prats et al. (2014) observed marked runoff and erosion as well as a slow vegetation recovery in the eucalypt burnt area of Ermida, although in a different eucalypt slope.

The distribution and concentrations of PAHs in soils have been found to depend on soil properties, with PAH levels being higher in soils and sediments that are rich in organic matter (Yang, 2000, Kim et al., 2003, 2011). Soil organic matter is considered one important factor in the environmental partitioning, accumulation and longevity of hydrophobic organic contaminants like PAHs (Means et al., 1980; Dzombak and Luthy et al., 1984; Nam et al., 2008). In general, we observed that soils and ashes with higher

OM also showed higher PAH concentrations (although not proportional). This is in line with the correlation analyses performed that suggest strong correlations between PAHs concentrations and OM levels for both soils and ashes, indicating that sequestration and incorporation of PAHs into burnt soils and ashes may also have been influenced by the OM content of particles. Kim et al. (2011) also reported strong correlations between total PAH content and organic matter content in burnt soils. Sojinu et al. (2011), however, did not find a correlation between total PAH content and total organic carbon values in burnt soil samples. This discrepancy can be explained by the fact that several other factors and, in particular, factors related to soil organic matter quality (composition) can also influence PAH accumulation in soils (Chiou et al, 1998; Wilcke, 2000).

4.5 Conclusion

This study provides some evidences of the influence of wildfires on the levels and composition of PAHs in forest soil. Concentration of PAHs in soils and ashes showed a strong non burnt soil-burnt soil-ashes gradient, with total concentrations up to four times higher in burnt soils than in unburnt soil in samples collected immediately after the fire, pointed to an extremely high incorporation of PAHs compounds in burnt soils during and after the forest fire. The LMW-PAHs (3-ring) and HMW-PAHs with 4-ring were the dominant PAHs groups in ashes and soils samples, however the 4-ring compounds accounted for the majority of PAHs concentrations in the unburnt soil, whereas ash and burnt soils were characterize by higher levels of 3-ring PAHs. The time-since-fire had a significant effect on PAHs levels burnt soil, which decreased considerably to the levels found in the unburnt soil and the composition pattern of PAHs became similar to the unburnt soil pattern. In addition, the temporal behavior of ashes were very similar to the burnt-soil. The ash-induced increase in the levels of PAHs in the soils was temporary because rain erosion remove ash from soil. The distribution patterns of PAHs displayed in the burnt soil and ash owing to the emission sources, implying the influence of biomass/coal burning on the distribution of PAHs in forest soils. This result is in line with the isomeric diagnostic ratios of PAHs, meaning that biomass/coal burning were the primary contributors to forest soils PAHs. Soils and ashes from pine hillslopes were, in general, enriched in PAHs compounds relatively to eucalypt areas. The temporal trend, pattern and isomeric ratios of PAHS clearly suggest that PAHs produced by forest fires seems to be adsorbed by ash and surface soil, which can be transported downstream by

soil/ash runoff. Leaching of ash as well as vaporization or biodegradation of LMW appears to be the main reasons for the rapid decrease in the levels of PAHs in burnt soil.

In summary, this study points out the importance of forest fires as an important natural source of PAHs. Notwithstanding, for an adequate assessment, monitoring and predicting the fire-induced pollution risks, more research is needed to further the knowledge and understanding of the off-site effects concerning the PAHs contamination by forest fires.

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Chapter V

Assessment of the toxicity of ash-loaded runoff from a recently burnt eucalypt plantation

5. Assessment of the toxicity of ash-loaded runoff from a recently burnt eucalypt plantation

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Abstract

Although wildfires are identified as an important source of polycyclic aromatic hydrocarbons (PAHs) and PAHs are well-known for their pernicious properties, the toxicity of runoff from recently burnt areas has received little research attention. This knowledge gap was addressed here through laboratory assays in which four aquatic species from distinct trophic levels were exposed to different dilutions of ash-loaded runoff. The runoff was collected in a recently burnt eucalypt stand in north-central Portugal on two occasions, immediately after the wildfire and about one year later. The total PAH load was about four times higher at the first than second sampling occasion (1194 vs. 352 ng l^{-1}) but even the latter value was considerably higher than those reported by prior studies on burnt areas. In addition, the two runoff samples differed noticeably in PAH composition, with a clear predominance of naphthalene in the second sample. Both runoff samples produced significant inhibitory effects on the three species representing the lower trophic levels, i.e. the bacteria *Vibrio fischeri*, the algae *Pseudokirchneriella subcapitata* and the macrophyte *Lemna minor*. The invertebrate *Daphnia magna* was not significantly affected but chronic tests are needed to discard the probable propagation of toxic effects from the lower trophic levels. Surprisingly, the runoff collected one year after the wildfire was the most toxic to *V. fischeri*, *P. subcapitata* and *L. minor.* Possibly, this was due to predominance of naphthalene in this sample. Surely, however, this demonstrated that detrimental off-site effects of wildfires are not necessarily limited to the immediate post-fire situation.

Keywords: Wildfire impacts, Ecotoxicological effects, Polycyclic aromatic hydrocarbons (PAHs), Ash-loaded runoff, Eucalypt, Aquatic species.

5.1 Introduction

Although wildfires are now widely recognized as a natural phenomenon in Mediterranean regions, present-day fire regimes strongly reflect human activities, in particular the widespread planting of highly flammable tree species in combination with land abandonment, resulting amongst others in an increase in fuel load (e.g. Lloret 2004; Moreira et al. 2009; Carmo et al. 2011; Shakesby 2011). In Portugal, wildfires are particularly problematic since have been affecting large areas in the past three decades, on average some 100,000 ha per year and over 400,000 ha in a dramatic year like 2003 (Pereira et al. 2005). Fire frequency in Portugal is also not expected to decrease in the foreseeable future, not just because of the likely increase in fire-propitious meteorological conditions due to climate change but also because of the nature of the country's forestry activities (Pereira et al. 2006; Fischlin et al. 2007).

Wildfires are an important societal and environmental concern worldwide, since their adverse effects are many-fold and include public safety and health, economic damages and costs (through fire prevention and fighting), air and water pollution, land-use sustainability and biodiversity. In the case of surface water pollution - the topic of this study - it is well documented that wildfires, through their direct effects on vegetation cover and soil properties, can lead to considerable changes in hydrological processes and the associated transport of sediments, nutrients and pollutants to downstream aquatic and flood zone ecosystems and man-made constructions (Shakesby and Doerr 2006).

Whilst in general wildfire effects are much better studied with respect to runoff amounts than their physico-chemical composition (Shakesby and Doerr 2006), fire-induced pollution of surface water by polycyclic aromatic hydrocarbons (PAHs) has been overlooked till quite recently (Olivella et al. 2006; Vila-Escalé et al. 2007; Schäfer et al. 2010). In a nutshell, the principal findings of these recent studies were that: (i) the export of PAHs from burnt areas into downstream water bodies could be increased substantially (by at least a factor 3); (ii) this effect was short-lived, dependent on distance to the burnt area; (iii) it did not produce a health risk since observed total PAH concentrations were below the limit for drinking water established by the European Community.

PAHs are pyrolytic substances that are classified as priority pollutants by the United States Environmental Protection Agency (USEPA 2002a) for their well-known toxic, mutagenic and carcinogenic properties as well as their environmental persistence and tendency for bioaccumulation along the food chain (ATDSR 1995; IARC 1998; Boström et al. 2002). PAHs have been found to produce a wide range of ecotoxicological

effects in a diverse suite of organism, including microorganisms, terrestrial plants, amphibians, reptiles and mammals (e.g. Wang et al 1993; Long et al. 1995; Delistray 1997; Rappaport et al. 2004; Cachot et al. 2006; Hellou et al. 2006). The reported effects involved acute toxicity, negatively affecting survival, growth and/or metabolic activity, as well as developmental and reproductive toxicity, enhancing DNA mutation, cancer formation and acting as endocrine disruptors (Delistray 1997; Clemons et al. 1998; Hellou et al. 2006). In spite these adverse effects of PAHs are well-established, the role of PAHs seems to have been disregarded in prior studies that showed noticeable consequences of wildfires on aquatic biota, including periphyton, macroinvertebrates and fish (e.g. Earl and Blinn 2003; Minshall 2003; Lyon and O´Connor 2008).

The present study addressed, first and foremost, the lack of information on the toxic effects of runoff from burnt areas. Furthermore, to the best of our knowledge it is the first assessment of PAH loads of surface water in recently burnt areas in Portugal. Both research gaps are particularly pertinent, now that the EU Water Framework Directive (WFD) is in its implementation phase and the need exists to duly identify diffuse sources of contamination of aquatic systems. The specific objectives of this study were to assess: (i) the ecotoxicological effects of runoff from a recently burnt eucalypt plantation in north-central Portugal, both immediately after the wildfire and one year later, using four aquatic species from different trophic/functional levels (*Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Lemna minor* and *Dapnhia magna*); (ii) the PAH loads of the runoff collected at the two above-mentioned sampling occasions; (iii) the relationships of the ecotoxicological effects on the four species with the observed PAH loads, also addressing the possible confounding role of nutrient availability.

5.2 Materials and methods

5.2.1 Study area and site

The study area is located near the hamlet of Colmeal, on the border of the municipalities of Góis and Arganil, in the Coimbra District of north-central Portugal (40º 08' 46'' N, 7º 59' 35'' W, 500 m asl; Figure 20). The area consisted of some 70 ha that were consumed by a wildfire on August 24 2008, and it was selected to study post-fire on soil erosion in the framework of the EROSFIRE-II project. The climate of the study area is of a transitional Atlantic-Mediterranean type, with wet winters and dry summers. According to the available information (in map format: APA, 2011), the mean annual

temperature is between 10 and 12.5 ^{o}C, whereas the average annual rainfall is between 1,400 and 1,600 mm. The geology of the area is composed of pre-Ordovician schists and greywackes (Ferreira 1978; Pimentel 1994), which have given rise to shallow soils that typically correspond to Humic Cambisoils (Cardoso et al. 1971, 1973). Prior to the wildfire, the study area was predominantly covered by fast-growing eucalypt plantations (Eucalyptus globulus Labill.) for paper pulp production, with the remaining 25 % or so consisting of Maritime Pine stands (*Pinus pinaster*).

Within the burnt area, five hill slopes were each instrumented with an unbounded slope-scale plot for monitoring post-fire erosion from late August 2008 onwards (Fernandes et al. 2010). One of these slopes was selected for the present study as being representative of the area's predominant land cover as well as of the prevalent, moderate-to-low fire severity. Fire severity was surveyed for the burnt area as a whole, by photography and visual inspection, from several look-out points, of the degree of the tree canopy consumption by the wildfire in the various forest stands (see also Maia et al. 2012). This involved distinguishing between light-colored patches where canopies were scorched only (low severity) and dark-colored patches where canopies were fully consumed (and thus, the ash-covered ground surface gave rise to the dark color; medium severity). All the stands within the burnt area presented a mixture of light and dark-colored patches, mostly with a clear predominance of the former. At the five instrumented hill slopes, fire severity was then assessed in more detail along a transect from the bottom to the top of the slope. This involved describing the degree of the tree canopy consumption of 10 randomly selected trees (partial vs. total) as well as the degree of the litter consumption (partial vs. total) and the color of the ash (black *vs.* grey) at 5 plots of 50x50 cm. In the case of the present study site, the prevalence of partially-consumed tree canopies, the total consumption of the litter layer and the black color of the ashes confirmed the moderate-to-low severity (see e.g. Shakesby and Doerr 2006; Keizer et al. 2008).

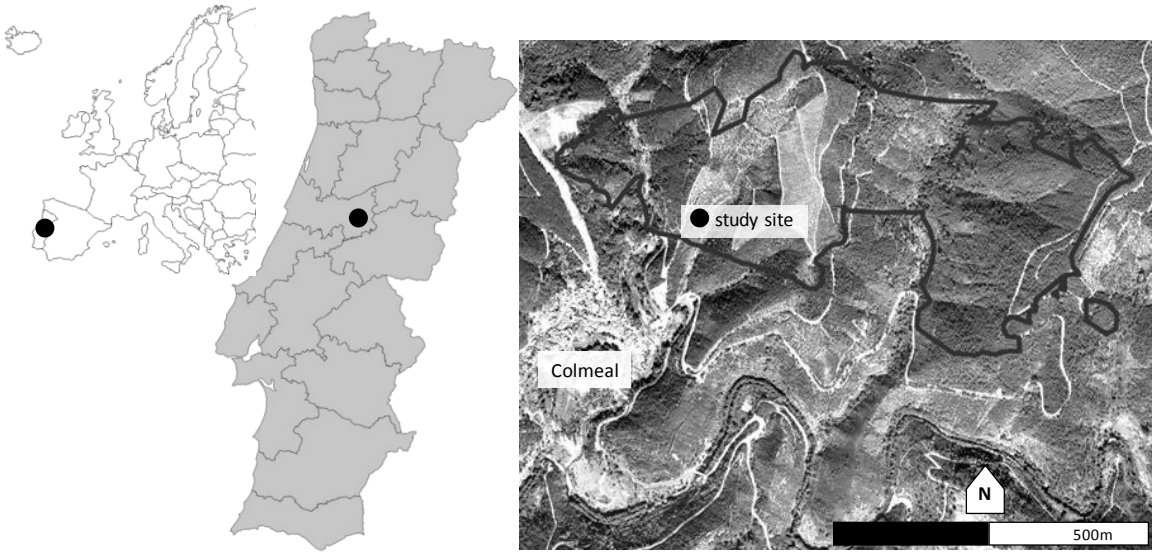

Figure 20 – Location of the Colmeal study area and selected eucalypt plantation instrumented with a slope-scale erosion plot (back dot)

5.2.2 Sampling procedures

At the selected slope, overland flow was collected at two occasions, namely on October 10 2008 after the first significant rainfall event following the wildfire and about one year later, on September 23 2009. This was done by mixing, in roughly equal proportions, the overland flow that had collected in the four 60 l tanks connected to the plot´s four outlets, so to obtain at each sampling occasion a single composite sample of the overland flow from a contributing area of approximately 100 m^2. The sample was then divided over various plastic bottles of 5 l, which, following transport and filtering in the laboratory using glass-fiber filters (1.2 μm pore size; APHA-2005), were stored at -18 ºC till further processing. Also the filters were stored at -18 ºC prior to the analysis of their PAH contents.

5.2.3 PAH analysis

The PAHs included in this study were the sixteen that the United States Environmental Protection Agency (USEPA) identified as priority contaminants. They were the following: naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene

(PYR), benz(a)anthracene (BaA), chrysene (CHR), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IND), dibenz(a,h)anthracene (DBA) and benzo(g,h,i)perylene (BGP).

The runoff samples were filtered using glass fiber filters a (GF/C, 1.2 μm) to analyze the dissolved and particulate PAH loads separately. The dissolved PAH concentrations of the filtered runoff were analyzed using solid phase micro-extraction (SPME) in a 100 μm poly-dimethysiloxane (PDMS) that was used as the absorbing material. After the extraction, the fiber was desorbed in a gas chromatograph (GC) (Varian CP-3800), with a split/splitless injector. The GC was coupled to a mass spectrometer Ion Trap Saturn 2200 (GC-MS) for the identification of the PAHs. The analytical procedure was validated by doping the sample with standards of the 16 PAHs. The recovery rates of the individual PAHs ranged from 70% to 117%. The detection limits (DL) were between 0.21 and 7.31 ng l^{-1}. The results presented here for each sample were the averages of two independent replicates.

Following drying of the filters at room temperature until constant weight, the particle-bound PAHs were extracted with acetone using microwaves high pressure and their concentrations were determined using the same analytical procedures as describe above.

5.2.4 Ecotoxicological assays

The ecotoxicological assays were carried out with four aquatic species from different trophic/functional levels that are commonly used for that purpose. They are the bacteria *Vibrio fischeri*, the green algae *Pseudokirchneriella subcapitata*, the macrophyte *Lemma minor*, and the invertebrate *Daphnia magna*. All four species were exposed to various dilutions of filtered (F) and unfiltered (UF) runoff. All ecotoxicological tests fulfilled the validity requirements established in their respective guidelines (AE 1998; OECD 1998, 2006a, 2006b).

Culture conditions of test species

Unialgal batch cultures of *P. subcapitata* were maintained in 250 ml erlenmeyer with 100 ml of Marine Biological Laboratory medium MBL sterilized Woods Hole Culture (Stein 1973) in an incubator chamber, with controlled temperature (20±2ºC) and photoperiod (16^{L}h:8^{D}h) and with light provided by cool-white fluorescent lamps. To start

new cultures, algae were harvested in the exponential growth phase (5-7 days old) and then inoculated into fresh medium.

The macrophyte *L. minor* was collected in a 250 ml erlenmeyer properly covered with cotton wrapped in gauze to minimize evaporation and accidental contamination, and was maintained in laboratory conditions in Steinberg medium (OECD 2006a) during eight weeks prior to testing. Culture *L. minor* were renewed 2 times per week and maintained in an incubator chamber, with controlled temperature (20±2ºC) and photoperiod (16^{L}h:8^{D}h) and light intensity of about 6500 lux.

Monoclonal cultures of *D. magna* (clone A, sensu Baird et al. 1989a) were continuously reared under a temperature of 20±2ºC and 16^{L}h:8^{D}h cycle, in synthetic ASTM hardwater medium (ASTM 1996) supplied with an organic additive extracted from the algae *Ascophyllun nodosum* (Baird et al. 1989b). Cultures were renewed every other day and the organisms fed with *P. subcapitata* (cyclically cultured in Woods Hole MBL medium according with Stein 1973) at a rate of 3.00 x 10^5 cells ml^{-1}.

Luminescence inhibition test with *V. fischeri*

The luminescence inhibition test (Microtox test) was performed according to the standard protocol (AE 1998). This involved measuring the decrease of bacterial luminescence following exposure to a series of dilutions of the filtered and unfiltered runoff, involving a single replicate per dilution. For the 81.9% basic test, the highest concentration tested was 81.9%. Bacterial luminescence was measured 5 and 15 min after the bacteria had been transferred to the vials samples.

Growth Inhibition test with *P. subcapitata*

Algal growth assays were conducted according to USEPA (2002b) and OECD (2006b) guidelines. The algae were exposed during 96 h period to several dilutions of filtered and unfiltered runoff water (12.5, 25.0, 50.0, 75.0 and 100.0%) in MBL medium. To discard any potential effects due to the possible lack of nutrients in highest concentrations tested, assays were performed without nutrients (100%) and with addition of nutrient according the formulation of the MBL medium (100%+N). Assays were performed for three replicates of each treatment plus the control in 100 ml glass vials containing 40 ml of test medium, under constant agitation (≈ 100 rpm in an orbital shaker), in the same conditions of algal cultures, with an initial cell density of approximately 104 cells ml^{-1}. At the end of the bioassay, the algae cell density (counting of cells on a microscope Olympus CKX41 using a Neubauer chamber), was determined

as a biomass parameter (APHA 2005). The endpoints growth rate (GR; day^{-1}) and percentage of growth inhibition (% I) were calculated from the cells density measurements.

Growth inhibition test with *L. minor*

The growth inhibition test was conducted according to USEPA (2002b) and OECD (2006a) guidelines. The macrophytes were exposed during 7 days to several dilutions of filtered and non-filtered runoff water (12.5, 25.0, 50.0, 75.0 and 100.0%) in Steinberg medium. In order to exclude any potential effects from nutrient deficiency promoted in the highest concentrations tested, assays were performed without nutrients (100%) and with addition of nutrient in the formulation of the Steinberg medium (100%+N).

Assays were performed for three replicates of each treatment plus the control, and colonies consisting of 3 visible fronds were transferred to 100 ml of testing medium from the inoculum culture and randomly assigned to the test vessels. The number of fronds and colonies was the same in each test vessels (9 fronds per vessel). The test conditions were the same as the culture conditions. The growth rate (GR; day^{-1}) and percentage of growth inhibition (% I) were determined measuring frond number and dry weight at the beginning and after 7 days of exposure.

Reproduction test with *D. magna*

Chronic reproduction assays with daphnids followed OECD (1998) and USEPA (2002b) guidelines. Runoff samples were tested at several dilutions (12.5, 25.0, 50.0, 75.0 and 100.0%) performed with ASTM. All the tests started with newborns ageing less than 24 h, born in the bulk cultures between the 3^{rd} and the 5^{th} brood, in order to minimize maternal effects (Barata and Baird 1998). For each treatment and control, 10 individual replicates were exposed during 21 days, in 50 ml glass vials, whose test medium was renewed every other day. The test conditions were the same already described for the maintenance of the daphnids, and the animals were fed every two days with *P. subcapitata*, at a rate of 3.00×10^5 cells ml^{-1}, supplied with an organic additive. Animals were daily observed for mortality and offspring production being the neonates counted and discharge. The endpoints recorded were survival, growth, fecundity (reproductive output), the age at first reproduction (AFR) and the number of broods. The body size of females was estimated immediately after the release of the first brood, and

at the beginning and at the end of the test, by extrapolation from the moult exopodite length (Pereira et al. 2004), allowing the calculation of the somatic growth rate of parent females (SGR, day^{-1}) (Burns 2000). All measurements were made under stereoscope (Olympus SZX9). Fecundity, survival and age at each brood release were integrated for the calculation of the rate of population increase (r, day^{-1}) using the Euler-Lotka equation. The Jackknife technique was used to calculate the standard deviation for r (Meyer et al. 1986).

5.2.5 Nutrient analysis

Nutrient analyses were carried out first and foremost to assess if nutrient limitations could interfere with the ecotoxicological results for *P. subcapitata* and *L. minor*, i.e. to address the possibility that these two species exhibited growth inhibition due to lack of available nitrogen (N) and phosphorous (P) rather than due to ecotoxicological effects. This potential effect of nutrient limitations was especially of interest in the case of the undiluted runoff samples, as dilutions were made with standard solutions with adequate nutrient levels. The concentrations of un-ionized ammonia (NH_3-N, i.e. the most toxic form of nitrogen for aquatic organisms (Koukal et al. 2004); Nessler method), nitrate (NO_3^-; cadmium reduction method), nitrite (NO_2^-; calorimetric method) and soluble orto-phosphate (PO_4^{3-} ; ascorbic acid method) were measured.

The nutrient concentrations of the unfiltered and filtered samples of both sampling periods are given in Table 14. All NO_3^- and NO_2^- values were below their detection limits (0.10 and 0.01 mg l^{-1}, respectively), whilst the NH_3 values only exceeded detectable amounts immediately after the fire. The PO_4^{3-} values did not only change by filtering but also with time-since-fire. Both changes were of the same sign as well as order of magnitude, corresponding to reductions by 60-65%. In the case of the NH_3 values for October-2008, the reduction by filtering was less pronounced, amounting to about 15%.

The nutrient concentrations in both filtered and unfiltered runoff were considerably below the levels of available N and P that provide optimal growth conditions for either *P. subcapitata* (OECD (2006b): 3.93 mg N l^{-1}; 0.29 mg P l^{-1}) or *L. minor* (OECD (2006a): 83 mg N l^{-1}; 23 mg P l^{-1}). Therefore, an additional treatment was included in the experiments with both species, as also done by e.g. Moreira-Santos et al. (2004). It involved the addition of nutrients to the undiluted runoff samples. It should be noted, though, that *L. minor* can grow under a markedly wide range of nitrogen and phosphorous concentrations (Landolt and Kandeler 1987). According to these authors, the minimal

nutrient levels for *L. minor* to achieve half of its maximum growth rate are 0.07 mg N l^{-1} and 0.0034 mg P l^{-1}, whereas the maximum levels that *L. minor* can tolerate are 375 mg N l^{-1} and 154 mg P l^{-1}.

Table 14 – Nutrient concentrations of filtered and unfiltered runoff collected in October 2008 and September 2009 from a recently burnt eucalypt plantation.

Nutrients (mg l^{-1})	**October 08**		**September 09**	
	Unfiltered	**Filtered**	**Unfiltered**	**Filtered**
PO_4^{3-}	1.91	0.68	0.64	0.26
NO_3^{--}- N	<0.10	<0.10	<0.10	<0.10
NO_2^{-}- N	<0.01	<0.01	<0.01	<0.01
NH_3- N	1.20	1.00	<0.10	<0.10

5.2.6 Data analysis

The effect concentration that produced a reduction of the measured endpoints of the test organisms with 50% - the so-called EC_{50} – was estimated using a special-purpose software in the case of *V. fischeri* (Microtox Omni™, version 4.3.0.1; AE 1998), and using Statistica 8.0 in the case of the other three test species. In Statistica, EC_{50} and its 95% confidence limits were estimated by non-linear regression using the logistic equation: $v_{2i} = c / (1 + (v_{1i} / EC_{50})^{**}b$, where: v_{2i} is growth rate in the case of *P. subcapitata* and *L. minor*, and somatic growth rate and rate of population increase in the case of *D. magna* at concentration i of the runoff sample; c is the maximum value within the control treatment; v_{1i} is the concentration i of the runoff sample; b is the slope of the line fitted through the data using the least squares method.

One-Way ANOVA was applied to test whether there existed significant differences, at α=0.05, in (somatic) growth rate (*P. subcapitata*, *L. minor*, *D. magna)* and the rate of population increase (*D. magna*) amongst the control treatment and the different dilutions of the runoff samples (Zar 1999). This was done using Sigmaplot 11.0, and included verification of the underlying assumptions of normality and homoscedasticity with the Shapiro-Wilk test and the Levene Median test, respectively. In the case of significant differences, Dunnett´s test was employed to test which treatment were significantly different to the control treatment (α=0.05), and this information was then used to

determine the lowest concentration of the runoff sample revealing an effect on species performance (LOEC).

The null hypothesis that nutrient limitations did not significantly (α=0.05) affect the growth rates of *P. subcapitata* or *L. minor* in the undiluted runoff samples (see 5.2.5.) was tested using Student´s t-test, as it concerned a contrast between two treatments ("100%" vs. "100%+N"). This was done with Sigmaplot 11.0 and, as before, included the verification of the underlying assumptions of normality and homoscedasticity.

5.3 Results and discussion

5.3.1 PAHs concentrations

The total loads of the 16 selected PAHs (ΣPAHs) dropped markedly between October 2008 and September 2009 (Table 15). Presumably, this reduction was first and foremost due to a reduced availability of ash deposits, as was also indicated by Olivella et al. (2006). In the case of the dissolved phase the decrease was about half, whereas in the case of the particulate phase it was almost three quarters. As a result, the predominance of the particulate over the dissolved fraction (reflecting the high affinity of PAHs for suspended particles) was less pronounced one year than immediately after the wildfire (i.e. with a factor 6 vs. 12). Such a decrease in the particulate/dissolved ratio with time-since-fire was also suggested by the results of Vila-Escalé et al. (2007), dropping from 95 to less than 2 between 12 and 445 days after fire. Unlike in the present case, however, it reflected first and foremost a reduction in the total concentrations of the particulate phase (from 369 to 7.1 ng l^{-1}), since the total dissolved concentrations were basically the same at these two sampling dates (3.9 vs. 4.3 ng l^{-1}). The discrepancy with the present study could be due the different sampling objects, i.e. the water samples of Vila-Escalé et al. (2007) were collected in a depositional pond at the downstream limit of a burnt catchment (in north-east Spain). This difference in sampling universe could also contribute to the markedly lower total concentrations in Vila-Escalé et al. (2007) than in this study, for both the dissolved and particulate fraction. A further difference in sampling object could also help explain why the October-2008 values differed even more from the total values obtained by Olivella et al. (2006) after the first, heavy post-fire rainfalls in two other burnt watersheds in north-east Spain (12 PAHs, dissolved + particulate phase: 0.3-37 ng l^{-1}). Namely, Olivella et al. (2006) not only studied riverine water samples but these samples also revealed a considerable dilution effect due to the rainfall.

Table 15 – PAHs concentrations in ng l^{-1} [mean ± standard deviation (n=2)] of the dissolved and particulate phases of runoff samples collected in October 2008 and September 2009 at a recently burnt eucalypt stand.

PAHs	Rings	October 2008		September 2009	
		Dissolved phase (ng l^{-1})	Particulate phase (ng l^{-1})	Dissolved phase (ng l^{-1})	Particulate phase (ng l^{-1})
NAP	2	14.8 ± 0.3	284 ± 105	24.5 ± 1.6	137 ± 6
ACY	3	<DL	343 ± 116	<DL	<DL
ACE	3	<DL	<DL	<DL	<DL
FLU	3	5.32 ± 0.35	41.5 ± 7.81	<DL	30.9 ± 0.1
PHE	3	<DL	42.4 ± 11.0	1.68 ± 0.18	37.7 ± 0.3
ANT	3	<DL	<DL	<DL	<DL
FLT	4	<DL	10.8 ± 0.6	1.22 ± 0.04	8.00 ± 0.61
PYR	4	1.86 ± 0.45	11.6 ± 2.8	1.21 ± 0.02	3.35 ± 0.16
CHR	4	14.6 ± 2.8	81.6 ± 10.5	14. 3 ± 1.0	74.6 ± 0.8
BaA	4	53.4 ± 20.1	290 ± 17	<DL	<DL
BbF	5	<DL	<DL	0.98 ± 0.08	1.94 ± 0.18
BkF	5	<DL	<DL	1.99 ± 0.16	4.78 ± 0.43
BaP	5	<DL	<DL	<DL	<DL
DBA	5	<DL	<DL	<DL	<DL
BCP	6	<DL	<DL	<DL	3.38 ± 0.11
IND	6	<DL	<DL	<DL	4.42 ± 0.29
∑PAHs		**90.0**	**1104**	**45.9**	**306**

<DL stands for below detection limit.

The composition pattern of PAHs by ring size revealed a more heterogeneous make-up for the dissolved than particulate phase, especially immediately after the wildfire (Figure 21). The 4-ring PAHs (MMW - Medium Molecular Weight) clearly dominated the dissolved phase in October 2008 (approx. 80%), whilst the 3-ring PAHs were underrepresented in the dissolved phase at both sampling occasions (4-6%). High Molecular Weight PAHs (HMW: 5 or 6 rings) were not detected in the October-2008 sample but amounted to approximately 5% in the September-2009 sample, in both the dissolved and particulate phase. Also the 2-ring PAHs revealed consistently higher contributions one year than immediately after the wildfire, even becoming the predominant fraction of the dissolved as well as particulate phase with roughly 50%. Possibly, the predominance of naphthalene in the PAHs profiles one year after the fire

derived from intrinsic factors, like biogenic synthesis (Meire et al. 2008), in combination with its elevated solubility. This could explain the marked increase in naphthalene's concentrations in the dissolved phase, whilst the concurrent decrease in the particulate phase would reflect the reduced availability of ashes. Also, "fresh" inputs of naphthalene by rainfall could play a role (Brun et al. 2004). The present results were quite different from those of Vila-Escalé et al. (2007), both for the dissolved and particulate phase. In the case of the dissolved phase, the 3-ring PAHs were the dominant fraction in the samples of Vila-Escalé et al. (2007) during the first 122 days after the fire, contrary to what was the case in this study. Also, the HMW PAHs were found by Vila-Escalé et al. (2007) from the first sampling date onwards. The same was true for the particulate phase. A further difference regarding the particulate phase was that the 4-ring PAHs clearly dominated over the entire period studied by Vila-Escalé et al. (2007). In the study of Olivella et al. (2006) in which the dissolved and particulate phase were analyzed together, the 3-ring PAHs tended to predominate after the first post-fire rainfall. This would agree well with the results of Vila-Escalé et al. (2007) for the dissolved phase, assuming that the dilution effect caused by rainfall observed by Olivella et al. (2006) affected especially the particulate phase.

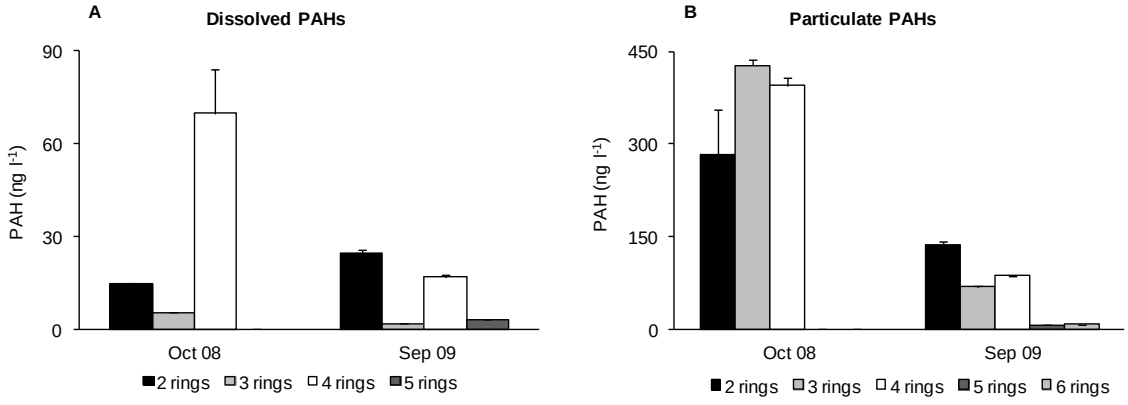

Figure 21 – The composition pattern of PAHs by ring size in dissolved phase (A) and particulate phase (B) in runoff water samples collected in October2008 and September 2009 from a recently burnt eucalypt plantation. Error bars represent standard error (n=2).

In terms of the individual PAHs, four compounds dominated the different samples (Table 15). Two of them – NAP (16-53%) and CHR (7-31%) - were principal components at both sampling occasions, whereas the two other – BaA (26-59%) and ACY (31%) - were dominant immediately after the fire but below the detection limit one year later. The dissolved phase revealed a striking shift in dominance with time-since-fire, from BaA

(59%) to NAP (53%). NAP (45%) also clearly dominated the particulate phase one year after fire. Immediately after the fire, however, NAP (26%) contributed to the particulate PAHs basically to the same extend as ACY (31%) and BaA (26%). Worth stressing is perhaps that BaA is one of the most potent carcinogenic PAHs. As to be expected from the above-mentioned differences in ring-based composition, the results presented here were markedly different from those of Vila-Escalé et al. (2007). A point of agreement, however, was that NAP was also one of the principal compounds of the dissolved phase immediately after the fire studied by Vila-Escalé et al. (2007). In the case of Olivella et al. (2006), PHE tended to be the principal compound after the first post-fire rainfall. Nonetheless, even their three highest concentrations (1-6 ng l^{-1}) were well below the present values (dissolved + particulate: 39-42 ng l^{-1}).

5.3.2 Toxicity tests

Luminescence inhibition test with *V. fischeri*

According to the Microtox basic test, the runoff collected immediately after the fire as well as one year later was highly toxic to *V. fischeri* (Figure 22; Table 16). In the case of the October-2008 sample, a toxic effect was only observed in the unfiltered runoff. By contrast, filtering did not avoid such a detrimental impact in the case of the September-2009 sample. Also the role of exposure time on the observed toxicity contrasted for the two sampling periods. Toxicity increased with increasing exposure time from 5 to 15 minutes for the (unfiltered) runoff collected immediately after fire, whilst the opposite was true for the (filtered) runoff collected one year later. The toxicity of PAHs for *V. fischeri* was also observed by Bihari et al. (2007), using PAH-contaminated seawater.

Table 16 – EC_{50} (%) values of the bacteria *V. fischeri* exposed to runoff samples collected at a recently burnt eucalypt stand in October 2008 and September 2009.

Sample	**Matrix**	**EC_{50} (%)**	
		5 min	**15 min**
October 2008	Unfiltered	10 (1-83)	7 (2-25)
	Filtered	NT	NT
September 2009	Unfiltered	ND	ND
	Filtered	3 (1-9)	8 (2-24)

The values between brackets represent the 95% confidence limits, and NT and ND stands for no toxicity and not determinate, respectively.

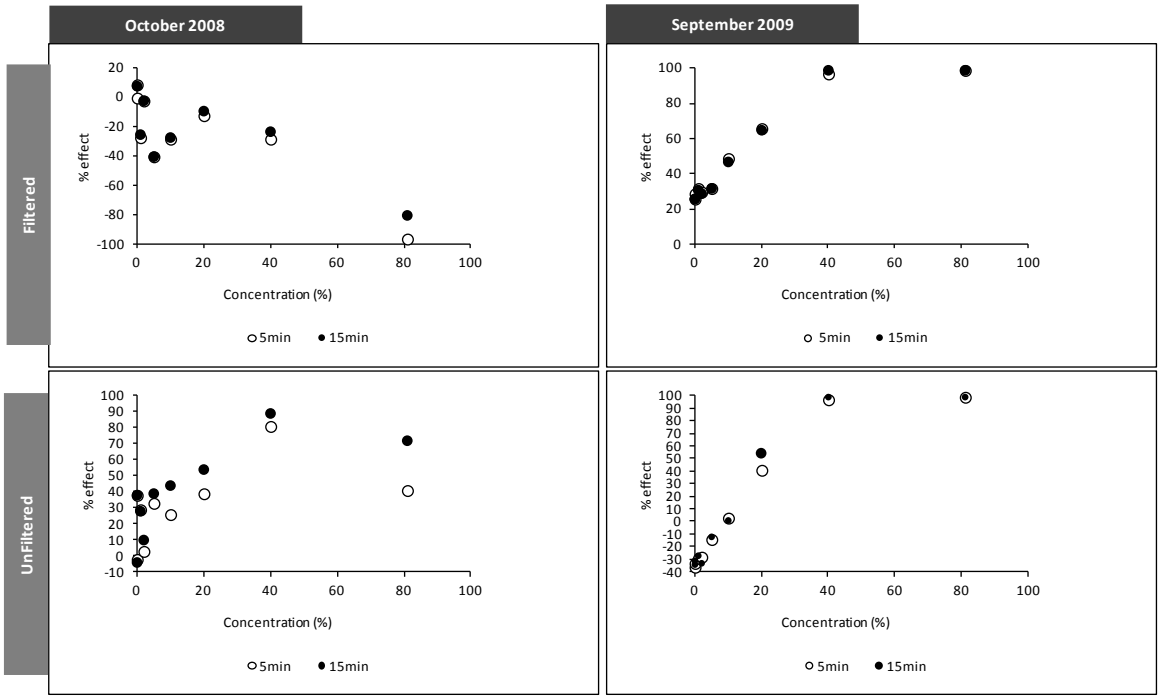

Figure 22 - Microtox luminescence inhibition of *V. fischeri* exposed to different concentrations of filtered and unfiltered runoff collected at a recently burnt eucalypt stand in October 2008 and September 2009.

Growth inhibition test with *P. subcapitata*

The growth rate of *P. subcapitata* was not only significantly but also strongly reduced (80-100%) by the undiluted runoff from the burnt eucalypt plantation (Figure 23; Table 17). This marked effect was observed independently of the filtering of the samples as well as of the time-since-fire. Even so, the sample of September 2009 appeared to provoke a stronger response than that of October 2008. This was suggested by the stronger growth reduction in the case of the undiluted samples (100 vs. 80%, the associated EC_{50} values being 60 vs. 90 (Table 18) but especially of the 25%-diluted samples (90-100 vs. 15-20%). Possibly, this tendency for an enhanced growth inhibition with time-since-fire was due to the above-mentioned changes in PAH composition, in particular the increased fraction of naphthalene, Namely, naphthalene is relatively water-soluble and, thus, easily available to species such as *P. subcapitata* (Kayal and Donnel 1995; Baumard et al. 1999a). Also the remaining dilutions of both sampling periods decreased the growth rate of *P. subcapitata*. This impact, while consistent, was minor (< 20%) and only statistically significant in the case of the filtered runoff collected in October 2008, giving a comparatively low LOEC value of ≤ 12.5%.

The addition of nutrients to the undiluted samples ("100%+N" treatment) attenuated significantly the growth rate inhibition of *P. subcapitata* (Figure 23; Student's t-test: p =

<0.001 - 0.03). This was true for the runoff of both sampling dates and for the filtered as well as unfiltered samples. In terms of the EC_{50} parameter, nutrient addition produced about 10 % higher values in the case of the October-2008 as well as the September-2009 samples (Table 18). The positive effect of additional nutrients was in line with the measured nutrient concentrations in the runoff samples (see Table 14), being below the optimal growth levels of *P. subcapitata* (OECD, 2006). Thus, adequate nutrient levels enhanced the species' tolerance to stressors, a phenomenon that was also observed by Moreira-Santos et al. (2004). Nonetheless, the compensatory role of the adequate nutrient levels was only effective to a certain degree, since the "100+N" treatments resulted in significantly lower growth rates than the control treatments in all four instances ($p<0.05$).

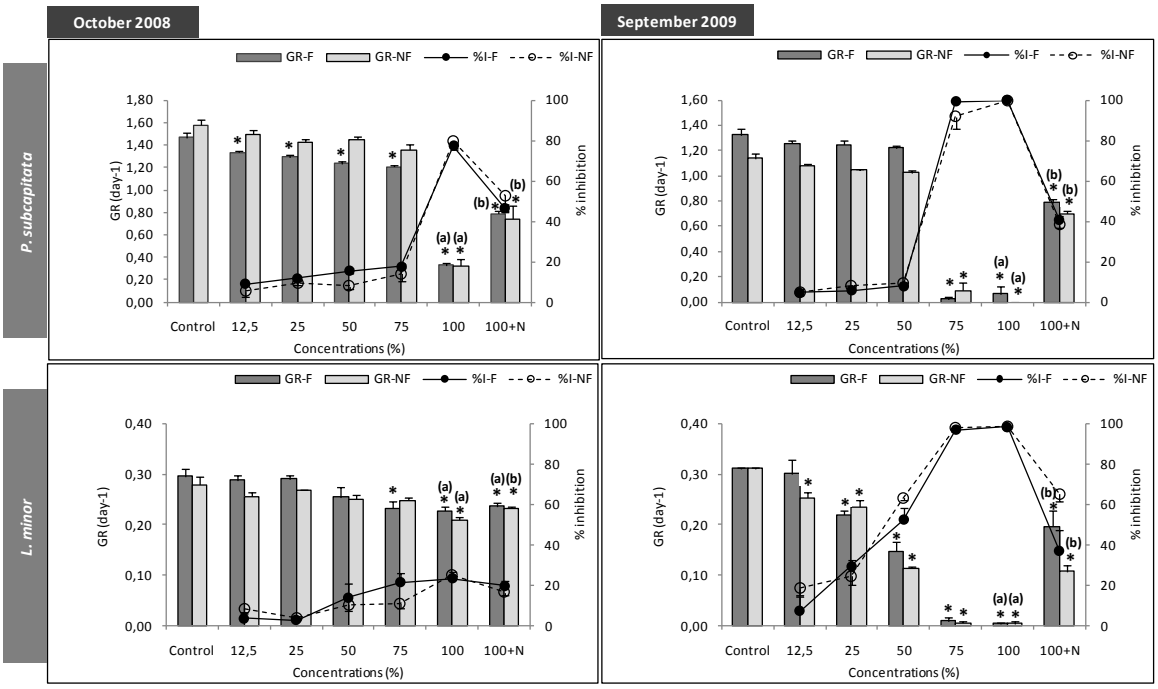

Figure 23 - Growth rates (GR) and % of inhibition (%I) for *P. subcapitata* and *L. minor* exposed to different dilutions of filtered (F) and unfiltered (NF) runoff collected at a recently burnt eucalypt stand in October 2008 and September 2009. Errors bars represent standard error, asterisks (*) indicate significantly differences from the control treatments, and the letters a and b indicates significant differences between the undiluted samples with additional nutrients (100+N) and without (100).

Table 17 – One-way ANOVA results and LOEC values (%; p<0.05) (%) for the growth rates (GR) of *P. subcapitata* and *L. minor*, and for the life-history endpoints of somatic growth rate (SGR) and intrinsic population increase (r) of *D. magna*, exposed to filtered (F) and unfiltered (UF) runoff collected at a recently burnt eucalypt plantation in October 2008 (Oct 08) and September 2009 (Sep 09).

Species	Endpoint	Sample		df	MS_{res}	F_{ratio}	H	p_{value}	LOEC
P. subcapitata	GR	Oct. 08	UF	6	$1.09e^{-2}$	62.283	-	< 0.001	100
			F	6	$1.95e^{-3}$	243.393	-	< 0.001	≤ 12.5
		Sep 09	UF	6	$3.17e^{-3}$	224.050	-	< 0.001	75
			F	6	$4.34e^{-3}$	229.177	-	< 0.001	75
L. minor	GR	Oct. 08	UF	6	$2.03e^{-4}$	7.848	-	< 0.001	100
			F	6	$4.98e^{-4}$	5.460	-	0.004	75
		Sep 09	UF	6	$2.31e^{-4}$	197.300	-	< 0.001	≤ 12.5
			F	6	$9.83e^{-4}$	48.029	-	< 0.001	25
D. magna	SGR	Oct. 08		6	-	-	17.349	0.008	ND
	r			6	-	-	31.086	< 0.001	ND
	SGR	Sep 09		6	$6.65e^{-5}$	2.324	-	0.045	ND
	r			6	-	-	16.929	0.01	ND

ND stands for not determinate.

Growth inhibition test with *L. minor*

L. minor exhibited a very similar response to the sample collected one year after the wildfire as *P. subcapitata* did (Figure 23; Tables 17 and 18). Namely: (i) the undiluted and 25%-diluted samples reduced the growth rate of *L. minor* to practically zero, whilst the greater dilutions had clearly less impact; (ii) filtering had no marked effect on this reduction in growth rate; (iii) addition of nutrients significantly attenuated these toxic effects but only partially, so that the response continued significantly different from that to the control sample. Notwithstanding these similarities, *L. minor* showed greater sensitivity to the September-2009 sample than *P. subcapitata*, as evidenced by the lower LOEC values (≤ 25 vs. 75%) as well as the lower EC_{50} values (38-53 vs. 60-74%).

By contrast, *L. minor* was less sensitive to the sample collected immediately after the wildfire than *P. subcapitata* in that the undiluted samples reduced the growth rate of *L. minor* to a markedly lesser – albeit still statistically significant - extent (approx. 20 vs. 80% for *P. subcapitata*). By implication, *L. minor* also revealed a stronger contrast in its response to the two sampling periods than *P. subcapitata*, as also evidenced by the

greater differences in EC_{50} values between the two sampling dates for both the filtered and unfiltered samples. In line with the explanation suggested in the previous section, *L. minor* would seem even more sensitive than *P. subcapitata* to the composition rather than to the total load of PAHs, in particular to the fraction of naphthalene presumably due to its comparatively high availability for uptake (Kayal and Donnel 1995; Baumard et al. 1999a). This could also explain why in the case of *L. minor* the attenuation effect of adding nutrients was only significant in the case of one of the two October-2008 samples as opposed to both September-2009 samples (Figure 23; Student's t-test: p = 0.001 – 0.462). On the other hand, the lack of a positive effect of nutrient addition could indicate that the growth *L. minor* was not as seriously nutrient limited as that of *P. subcapitata*, possibly because the species' marked tolerance to suboptimal nutrient concentrations as those measured in the undiluted runoff samples (Landolt and Kandeler 1987; OECD 2006a).

Table 18 – EC_{50} values (% dilution) and respective 95 %- confidence limits at the growth rates (GR) of *P. subcapitata* and *L. minor*, and for the life-history endpoints of somatic growth rate (SGR) and intrinsic population increase (r) in *D. magna*.

Species	Endpoint	Sample		EC_{50} (%)	
				Unfiltered	Filtered
P. subcapitata	GR	Oct. 08	100	90.3 (87.1-93.5)	90.8 (87.1-94.3)
			100+N	99.9 (95.4-104.5)	>100
		Sep 09	100	62.2 (58.2-66.3)	59.8 (53.3-66.3)
			100+N	74.0 (40.9-107.1)	69.7 (41.9-97.5)
L. minor	GR	Oct. 08	100	> 100	>100
			100+N	>100	>100
		Sep 09	100	41.8 (35.8-47.8)	40.8 (31.8-49.7)
			100+N	38.3 (24.8-51.8)	52.8 (11.7-93.8)
D. magna	SGR	Oct. 08		NT	NT
	r			NT	NT
	SGR	Sep 09		NT	NT
	r			NT	NT

The 100+N treatment involved the addition of nutrients, and NT stands for not toxic.

Reproduction test with *D. magna*

Unlike was the case for the other three test species of lower trophic levels, *D. magna* did not reveal significant inhibitory effects to either the runoff collected immediately after the wildfire or that collected one year later (Figure 24; Tables 17 and 18). As reviewed in ECHA (2009), it is well-documented that daphnids can be less sensitive to contaminated water than species of lower trophic levels. Possibly, the daphnids in this study were able to modify their energy allocation patterns to permit their maintenance and survival under more adverse conditions, as also suggested by Polishchuk and Vijverberg (2005) and Pieters and Liess (2006). Furthermore, the daphnids might have improved their fitness by extending their diet to include dissolved or suspended nutrients (Roche 1998; Nandini et al. 2005), particulate organic matter (Antunes et al. 2007a) and/or bacteria (Lampert 1987), especially the present study concern natural runoff samples (Peterson et al. 1978; Jürgens 1994; Langenheder et al. 2001; Degans et al. 2002; Xumang et al. 2011). Bioassays carried out with natural samples have frequently revealed positive rather than the expected negative responses on cladoceran life-history traits (e.g. Antunes et al. 2007a, 2007b; Abrantes et al. 2008, 2009; Marques et al. 2011). The October-2008 sample did, in fact, show some significant increases in the rates of somatic as well as population growth. They were more likely related to consumption of algae and/or particulate organic matter than of nutrient, as the nutrient concentrations were rather low (Table 14). The role of the particulate phase was also suggested by the fact that filtering significantly decreased population increase to levels similar to control conditions as well as by the fact that the October-2007 sample had a much higher particulate PAH load than the September-2009 sample. Besides to such compensatory phenomena, the lack of growth-reducing effects could simply be due to the relatively low PAH concentrations observed in this study. Namely, none of the 16 PAHs attained concentrations in either the filtered or unfiltered runoff samples exceeded 350 ng l^{-1}, whilst Lampi et al. (2006) and Alvarez et al. (2008) reported 48-h median lethal concentrations (48-h LC50) of individual PAHs for *D. magna* that ranged from 1,040 ng l^{-1} in the case of BGP and 699,000 ng l^{-1} in the case of PHE.

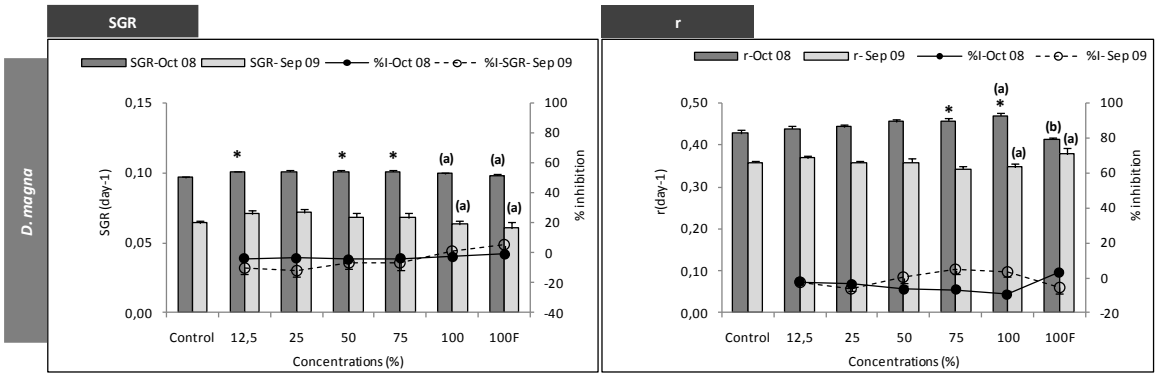

Figure 24 - Somatic growth rate (SGR, day^{-1}) and intrinsic rate of population increase (r, day^{-1}) of *D. magna* exposed to different concentrations of runoff water samples collected at a recently burnt eucalypt stand in October 2008 and September 2009, where "100F" stands for filtered undiluted sample. Errors bars represent standard error, asterisks (*) indicate significantly differenced from the control conditions (α≤0.05), and the letters a and b indicate significant differences between the filtered and unfiltered, undiluted samples 100F and 100, respectively; α≤ 0.05).

Overall discussion

The results of this study amply justify concerns about the off-site environmental impacts of wildfires, with three of the four test species revealing significant inhibitory effects when exposed to overland flow from a recently burnt area. Especially in the context of the ongoing implementation of the EU Water Framework Directive (WFD), recently burnt areas should thus not be ignored as potential point sources of pollution for downstream surface water bodies. While the present findings agreed with the decrease in PAH loads with time-since-fire reported by prior studies (Olivella et al. 2006; Vila-Escalé et al. 2007), they also evidenced that environmental risks are not necessarily limited to the immediate post-fire situation and, thus, would need to be monitored for more than at least one year.

For an adequate assessment and monitoring of fire-induced pollution risks, however, much research is needed to further the knowledge and understanding of various key aspects, related with the characteristics of the fire, the sediment transport processes through the hydrographic network, the biogeochemical cycle of the pollutants as well as their ecotoxicological effects. Worth mentioning in this respect is perhaps that some of the important limitations of the present work are now being addressed by a follow-up study, extending the sampling universe in terms of study sites, land-cover types, sampling objects (soil, ashes, sediments), sampling period and frequency as well

as broadening the ecotoxicological testing to include e.g. chronic bioassays with *D. magna.* Such chronic bioassays are expected to reveal that the fire-induced effects on organism of higher trophic levels (including humans) are first and foremost indirect, via the propagation of toxic effects through the food web by bottom-up mechanisms (Abrantes et al. 2008).

A major challenge for the follow-up work on the ecotoxicological effects of ash-loaded runoff will be to address the complexity of influencing factors, especially also for test species of the higher trophic levels due to bioaccumulation processes (e.g. Bícego et al. 2006). This was illustrated well by the present study in that it proved difficult to pinpoint the observed inhibitory effects to specific aspects of the PAH loads of the tested runoff samples. A first reason was that the samples, as seems to be typical in burnt areas (Olivella et al. 2006; Vila-Escalé et al. 2007), consisted of mixtures of multiple (predominant) compounds, so that the role of possible synergistic, antagonistic or additive effects between the various PAHs could not be precluded (Pardos et al. 1998). Second, the sample with the clearly highest PAH load – i.e. that collected in October 2008 after the first post-fire rains – appeared to inhibit the three species of the lower trophic levels to a lesser extent than the sample collected one year later. Third, filtering supposedly removed about 90% of the overall PAH load but this did not substantially attenuate the inhibitory effect of the runoff, except in one out of six cases (that of the October-2008 sample on *V. fischeri*). A possible explanation for the limited role of the particulate phase was that the PAHs were strongly absorbed to the particles and, thus, poorly “available” to the test species, especially since the particulate phase consisted to a large extent of charred organic matter (see Malvar et al. 2011). In a real-world situation, however, this particulate phase could be a chronic source of PAH contamination in depositional environments, also because PAHs are relatively stable compounds, especially also under anaerobic conditions (e.g. Mihelcie and Luthy 1988; Kim et al. 1999).

In line with the above-mentioned, any explanation for the most unexpected finding of this study - the greater effects of the runoff collected one year than immediately after the fire - can at best be tentative. An obvious factor that could be involved was naphthalene or, to be more specific, either the relative amounts of naphthalene in the total loads of dissolved and particulate PAHs (45-50 vs. <25%) or the absolute dissolved naphthalene concentration (25 vs. 15 ng l^{-1}). The importance of the dissolved naphthalene concentration would agree especially well with the fact that LMW PAHs (2-3 rings) and especially naphthalene are more water-soluble than MMW PAHs (4 rings), and, thus, more easily “available” to species like *P. subcapitata* and *L. minor* (Kayal et al.

1995; Baumard et al. 1999a, 1999b). Furthermore, LMW PAHs tend to produce a stronger acute toxicity than MMW or HMW PAHs (5-6 rings) (Law et al. 1997; Boochan et al. 2000). On the other hand, the concentrations reported here were well below the concentrations, at which individual LMW PAHs were found to inhibit growth and cell division in aquatic bacteria and algae (5-100 µg l^{-1}), let alone to impair their cell division and ultimately cause their death (0.2-10 mg l^{-1}) (Eisler 2000). This discrepancy highlights the possible existence of combined effects in the present samples with their highly variable mixtures of distinct PAHs. Such combined effects are notoriously difficult to predict on the basis of individual toxicities of indentified compounds (Pardos et al. 1998; Brack 2003; Wenzl et al. 2006).

5.4 Conclusion

The principal conclusions of this study into the ecotoxicological effects of overland flow collected at a recently burnt eucalypt stand in north-central Portugal immediately after a low-medium severity wildfire and one year later were the following:

(i) the total amounts of 16 priority polycyclic aromatic hydrocarbons (PAHs) in the collected runoff samples were high compared with the values of prior studies in burnt areas, probably due to differences in sampling objects (hillslope runoff in this study vs. riverine and depositional ponds);

(ii) the dissolved as well as particulate PAH loads were markedly higher following the first rainfall event after the wildfire than one year later, and also had noticeably different compositions in terms of ring-based as well as individual PAHs;

(iii) the runoff produced statistically significant and, in general, conspicuous inhibitory effects on three of the four test species, i.e. on the bacteria *V. fischeri*, the green algae *P. subcapitata* and the macrophyte *L. minor* but not on the invertebrate *D. magna* representing the highest trophic level;

(iv) surprisingly, the runoff collected immediately after the wildfire was less toxic to *V. fischeri*, *P. subcapitata* and *L. minor* than the runoff collected one year later, suggesting that total loads of PAHs can be less important than their composition, and clearly demonstrating that the off-site effects of wildfires are not necessarily limited to the period immediately after the fire.

(v) further research is urgently needed to provide a sound scientific basis for assessing, monitoring and predicting the risks of surface water pollution by recently burnt areas, including with respect to the “window-of-ecotoxicological-effects” by runoff from

recently burnt areas, the role therein of individual and mixtures of PAHs, and the propagation of toxic effects from the lower to the upper trophic levels.

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Chapter VI

General conclusions and future perspectives

6. General conclusions and future perspectives

The present section aims to provide an overview and integration of the main findings of each chapter (II-V), contributing with informative perspectives. The current discussion was achieved taking into account the key objectives of the thesis and it´s throughout progression.

Although fires are recognized as a natural phenomenon that contribute to shape the forest landscape and vegetation, especially in Mediterranean-type climate regions, like Portugal, present-day fire regimes reflects the human activities (e.g. country's forestry activities of widespread planting of flammable pine and eucalypt species and land abandonment) as well as climate conditions, resulting in the increased frequency, severity and extent of wildfires. Indeed, the environmental impacts of wildfires promote significant effects to the structure and functioning of the different ecosystem compartments. In particular, the effects of fire-induced release of contaminants, such as, polycyclic aromatic hydrocarbons (PAHs) and major and trace elements (metals), which are believed to be of a specific ecological, biological and/or human significance, due to their high toxicology, persistence and tendency to bioaccumulate, has been overlooked till quite recently, mainly in Portugal.

A key-issue of this study was: "can wildfires be a concerning diffuse source of contaminants to the soil and aquatic systems?" Hence, the present work is a contribution to clarify and evaluate the role of wildfires in the mobilisation of metals (V, Mn, Co, Ni, Cu, Cd, Hg and Pb) and PAHs and their off-site effects. As a way to fulfil these propose, an integrative approach was adopted. First, it was assessed the effects of a wildfire and the influence of time-since-fire (immediately after the fire and, four, eight and fifteen after the fire) on the mobilisation of metals (Chapter II and III) and PAHs (Chapter IV) from ashes and soils (soil system). It was also investigated the role of the two principal forest plantation in the north-western part of the Iberian Peninsula, i.e. eucalypt and maritime pine plantations in the levels of these contaminants in ashes and soils (Chapter II –IV). Finally, were assessed the toxic effects of the surface runoff from an eucalypt burnt area, collected immediately after the fire and one year later, to study the off-site effects of a wildfire on the aquatic system (Chapter V). To this end, the chemical levels of contaminants of the overland flow was focused on the PAHs and then an ecotoxicological assessment (to infer on the deleterious off-site effects on aquatic biota) was performed using four standard species representing different functional groups and

trophic levels: the bacteria *Vibrio fischeri*, the microalgae *Pseudokirchneriella subcapitata*, the macrophyte *Lemna minor* and the invertebrate, *Daphnia magna*. The combined strategy was chosen in order to avoid the gaps of sole chemical monitoring, since it only provides a measure of the contamination status of a given environmental compartment, disregarding the contaminants bioavailability, the occurrence of interactions between chemicals and the impairment expected for the ecosystem resulting there from. This approach by integrating the chemical assessment with the toxicity evaluation, allows for a holistic overview.

The present study confirmed and emphasizes the role of wildfires on the mobilisation of metals and PAHs (this one is are also produced) to the environment, acting thus as a source of environmental contamination as previously reported by other authors. Additionally this study also suggested that this contaminants mobilisation was not limited to the soil compartment, but their inflow into water systems, especially by ash-loaded overland flow, could also lead to serious and complex impacts on ground water and surface water quality as well as on aquatic biota. These detrimental off-site effects are particularly important during the initial post-fire period, although not necessarily limited to it.

The information resulting from the chemical assessment of ashes and soils has the potential to give some insights on environmental implications of wildfires. Understanding the dynamic of metals and PAHs on the environment is essential for ecosystem management and risks assessment of environmental pollution and sustainability. Their distribution in ecosystems is a key issue in environmental management and protection. An interesting point in our results was the different role of the wildfire in the behaviour of metals in soils. The measured concentrations of V, Mn, Ni, Cd and Pb (chapter III) were higher in burnt eucalypt soils than in unburnt soils, pointing to an input of these elements in burnt soils. In fact, it can be stressed that the ash layer deposited after the fire can act as an important source of this elements to the burnt soil. The levels of Co and Cu revealed no differences. However, 30 % of the Hg retained in the eucalypt soils was released from soil during the wildfire (corresponding to a loss of 1.0-1.1 g Hg Ha^{-1}), predominantly in the form of gaseous Hg(0) and in a much lesser levels in the form of particulate mercury. One of the main reason for this huge difference (Hg losses), result from the low volatilization temperatures of Hg species, which is of particular concern due to the relevance of the atmospheric comportment in the global Hg cycle. Indeed, Hg released from forest fires can have local, regional and global impacts. Taking this into consideration and the short-term study of the impacts of wildfire and subsequent rainfall event (four and fourteen weeks after the fire) on mercury

redistribution it was decided to present an individual paper research for Hg (Chapter II). As reported in Chapter IV, the levels of PAHs in the soils became significantly elevated by the wildfire, which seem to be influenced by the ash deposited in the topsoil. Additionally, the relatively more volatile PAHs with 3-4 rings were dominant in ashes and soils, reflecting the influence of PAHs production/emission from the biomass burning in forest soils, which was also confirmed by the isomeric diagnostic ratios. Furthermore, the 4-ring compounds accounted for the majority of PAHs concentrations in the unburnt soil, whereas ash and burnt soils were characterize by higher levels of 3-ring PAHs.

As before mentioned, the role of the ash layer as an important agent influencing the levels of metals and PAHs in burnt soils is supported by the enrichment of ashes comparatively to the underlying soils in all the contaminants and also by the fact that our results showed the same order of concentration between ashes and soils for the metals (Hg<Cd<Co<Ni<Cu<V<Pb<Mn). As for PAHs, this hypothesis is also reinforced by the similar PAHs composition pattern profile of ashes and burnt soil.

Our results showed three different patterns in the behaviour of studied elements in burnt soils with time-since-fire: (1) concentrations of Mn, Cd and PAHs declined abruptly after the first rainfall event (four months after the fire); (2) concentrations of V, Ni and Co increased during the first eight months; and (3) levels of Cu, Hg and Pb hardly changed during the studied period. Additionally, ashes revealed peak concentrations in metals and PAHs immediately after the fire, with a sharply decline four months later, which were more consistent and more pronounced than in burnt soils. These decreases could be explained by to the heavy rainfall and subsequent overland flow that have promoted the washout and leaching of these elements from soils and ashes during this initial period of the window-of-disturbance and would then imply the diffuse contamination of surface water and/or groundwater. Furthermore, vaporization or biodegradation can also contribute for the rapid decrease in the levels of PAHs in burnt soils. The different temporal variations of the metals in soil could be explain by their mobility, the nature of its association with organic matter, oxides and with other soluble species, soil ability to release the metal from the solid phase as well as soil structure and profile development. Regarding the two forest type, we found that ashes and soils from the pine hillslopes were consistently enriched in PAHs, Co and Ni relatively to the eucalypt areas. Interestingly, the opposite was observed for Hg concentrations. Additionally, this study also demonstrated that heavy rainfall episodes after the wildfire can play an important role in the mobilisation of those elements in the environment.

Chemical alterations in the forest soils following fire, as the ones pointed in this study may also alter microbial communities and the process they catalyze. In fact, the

accumulation of major and trace elements in forest soils can provoke toxic responses on soils microorganisms in terms of reduced microbial biomass, reduced carbon mineralization and disturbance of enzymatic activities. Microbial communities undergoing a metal stress, moreover, tend to change structure and diversity. This eventually affects the biogeochemical systems functions driven by these organisms, since soil microorganisms are important agents in the nutrient cycling and energy flow. Indeed, increased wildfire frequency and strong rainfall have a greater potential to reduce soil productivity by erosion and decrease soil periods for recovery of microbial mineralizing activities and to loss of ecosystem resilience in the long term. This way, understanding fire effects on soil processes and patterns is critical for long-term forest planning and management. In particularly, knowledge of fire impacts on contaminant, such as metals and PAHs is important because of the potential for persistent negative effects on soil fertility and productivity, especially when wildfires occur with a frequency or severity outside of the historic range of variation.

Overall, the results from this chemical assessment pointed out the role of wildfires, as well as rainfall in the mobilisation of metals and PAHs in the environment and that the risks of contamination posed by the wildfires are not limited to the in-situ burnt soils, but also includes surface and groundwater bodies within the downstream of a burnt area.

Another aim of the present work was the ecotoxicity assessment (Chapter V) to evaluate the off-site effects of wildfires. Once again, the chemical assessment of the runoff collected in a burnt area revealed high PAHs loads following the first rainfall event after the wildfire, with a clear decreased one year later. Likewise, a noticeable different composition in terms of ring-based as well as individual PAHs was found one year after the fire occurrence. Regarding the ecotoxicological assessment, both runoff samples induced different responses in the tested organisms: significant inhibitory effects were observed on the bacteria *Vibrio fischeri* as well as on the primary producers, the microalgae *Pseudokirchneriella subcapitata* and the macrophyte *Lemna minor*, while no noxious effects were observed for the invertebrate *Daphnia magna*.. This way, this integrated approach demonstrated that the risks of contamination are not limited to the on-site burnt soils. Hence, results of this study amply justify concerns about the off-site environmental impacts, especially in the context of the implementation of the Water Frame Directive (WFD), which established the obligation to characterize the various sources of point and diffuse pollution of water bodies. This way, recently burnt areas should not be ignored as potential point sources of pollution for downstream surface or groundwater bodies.

In summary, this study points out the importance of forest fires as an important natural source of metals and PAHs. Notwithstanding, for an adequate assessment, monitoring and predicting of the fire-induced pollution risks, more research is needed to generate new knowledge and understanding of the off-site effects concerning the metals and PAHs contamination by forest fires.

This study provides relevant information that could be helpful in future environmental decisions. Overall, findings of this study brings a new insight on the role of wildfire and subsequent rainfall in the mobilisation of metals and PAHs in ashes and soils from burnt forest plantations, which constitutes an important source of diffuse pollution. In addition, this study also emphasize the risks posed by these contaminants to downstream water bodies, with deleterious consequences for both chemical and biological conditions. In view of that, the findings of this study may be useful as starting point to design and implement future monitoring programs in burnt catchments as part of post-fire management plans.

However, some fundamental research questions remain unanswered and further knowledge is needed to better understand the on-site and off-site implication of wildfires, as well as the complexity of the influencing factors. In this respect, new integrative knowledge is being generated under the scope of follow-up studies conducted within our group (Earth and Surface Processes Team), which entails the comprehension of the physical processes, the chemical characterization and the assessment of the toxic effects on the ecosystems.